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(54) Title: COMPOUNDS FOR THE TREATMENT OF DISEASES INVOLVING COGNITIVE IMPAIRMENT, SUCH AS ALZHEIMER'S DISEASE, AND METHODS FOR IDENTIFYING SUCH COMPOUNDS

(57) Abstract: The present invention relates to compounds for use as a medicament in diseases involving cognitive impairment such as Alzheimer's disease. In particular, the present invention relates to antagonists of and translation inhibitory compounds of the gene encoding FPRL1, FPRL2, FPR1, GPR32, CMKLR1, C5R1, GPR44, GCGR, GLP1R, GLP2R, GIPR, VIPR1, SCTR, VIPR2, providing a reduction of the levels of amyloid-beta protein. The invention further relates to methods for identifying such agonists and translation inhibitory compounds and methods for diagnosing a pathological condition involving cognitive impairment.

COMPOUNDS FOR THE TREATMENT OF DISEASES INVOLVING COGNITIVE IMPAIRMENT, SUCH AS ALZHEIMER'S DISEASE, AND METHODS FOR IDENTIFYING SUCH COMPOUNDS

The present invention relates to compounds for use as a medicament in diseases involving cognitive impairment such as Alzheimer's disease. The invention in particular relates to antagonists and translation inhibitory compounds that reduce the levels of amyloid-beta protein. The invention further relates to methods for identifying such compounds and methods for diagnosing a pathological condition involving cognitive impairment.

Alzheimer's disease is a neurological disorder that is clinically characterized by the progressive loss of intellectual capacities: initially memory, and later on by disorientation, impairment of judgment and reasoning, commonly referred to as cognitive impairment, and ultimately full dementia. The patients finally fall into a severely debilitated, immobile state between 4 and 12 years after onset of the disease. Worldwide, about 20 million people suffer from Alzheimer's disease. The pathological hallmarks of Alzheimer's disease are the presence of extracellular amyloid plaques and intracellular tau tangles in the brain, which are associated with neuronal degeneration (Ritchie and Lovestone (2002)).

A small fraction of alzheimer's disease cases are caused by autosomal dominant mutations in the genes encoding presenilin 1 and 2 (PS1; PS2) and the amyloid-beta precursor protein (APP). It has been shown that mutations in APP, PS1 and PS2 alter amyloid-beta precursor protein metabolism such that more of the insoluble, pathogenic amyloid beta 1-42 is produced in the brain. Following secretion, these amyloid beta 1-42 peptides form amyloid fibrils more readily than the

amyloid beta 1-40 peptides, which are predominantly produced in healthy people. These insoluble, amyloid fibrils are then deposited in the amyloid plaques.

The amyloid beta peptides are generated from the membrane anchored APP, after cleavage by beta secretase and gamma secretase at position 1 and 42, respectively (Figure 1)(Annaert and De Strooper (2002)).

The gamma secretase can also cleave at position 40. In addition, high activity of beta secretase results in a shift of the cleavage at position 1 to position 11. Cleavage of amyloid-beta precursor protein by alpha secretase activity and gamma secretase activity at position 17 and 40 or 42 generates the non-pathological p3 peptide.

Beta secretase was identified as the membrane anchored aspartyl protease BACE, while gamma secretase is a protein complex comprising presenilin 1 (PS1) or presenilin 2 (PS2), nicastrin, Anterior Pharynx Defective 1 (APH1) and Presenilin Enhancer 2 (PEN2). Of these proteins, the presenilins are widely thought to constitute the catalytic activity of the gamma secretase, while the other components play a role in the maturation and localization of the complex. The identity of the alpha secretase is still illustrious, although some results point towards the proteases ADAM 10 and TACE, which could have redundant functions.

It has been shown that injection of amyloid beta fibrils in the brains of P301L tau transgenic mice enhances the formation of neurofibrillary tangles, placing the amyloid beta peptide on top of the neurotoxic cascade (Gotz et al. (2001)). Although no mutations in PS1, PS2 and amyloid-beta precursor protein have been identified in late onset ALZHEIMER'S DISEASE patients, the pathological hallmarks are highly similar to the early onset ALZHEIMER'S DISEASE

patients. Therefore, it is generally accepted that aberrant increased amyloid peptide levels in the brains of late onset Alzheimer's disease patients are also the cause of the disease. These increased levels of amyloid beta peptide could originate progressively with age from disturbed amyloid-beta precursor protein processing (e.g. high cholesterol levels enhance amyloid beta peptide production) or from decreased catabolism of the peptide.

Since the socio-economical impact of ALZHEIMER'S DISEASE is large, the need for an effective therapy is urgent. Because the cholinergic neurons are the first neurons to degenerate during ALZHEIMER'S DISEASE , levels of the neurotransmitter acetylcholine decrease, resulting in the progressive loss of memory. Therefore, the major current alzheimer's disease therapies are focused on the inhibition of the acetylcholinesterase enzyme, leading to an increased concentration of the acetylcholine. However, this therapy does not halt the progression of the disease.

Therapies aimed at decreasing the levels of amyloid beta peptides in the brain, are heavily investigated and will become very important. Most of these therapies are focused on the perturbed amyloid-beta precursor protein processing and target directly beta- or gamma secretase activity. However, targeting these proteins has not yielded any new drugs yet, because of the difficulty to find specific drugs and of suspected serious side effects.

The present invention provides the identification of compounds that reduce the level of amyloid-beta protein production, thus providing new drugs for the treatment of diseases involving cognitive impairment, such as Alzheimer's disease.

Further the present invention provides novel methods for identifying such novel drugs.

The novel drugs according to the present invention are antagonists of a polypeptide selected from the group consisting of formyl peptide receptor-like 1 (FPRL1, SEQ ID No:1 and 15); formyl peptide receptor-like 2 (FPRL2, SEQ ID No:2 and 16); formyl peptide receptor 1 (FPR1, SEQ ID No:3 and 17); G protein-coupled receptor 32 (GPR32, SEQ ID No:4 and 18); chemokine-like receptor 1 (CMKLR1, SEQ ID No:5 and 19); complement component 5 receptor 1 (C5a ligand) (C5R1, SEQ ID No:6 and 20); G protein-coupled receptor 44 (GPR44, SEQ ID No:7 and 21); glucagon receptor (GCGR, SEQ ID No:8 and 22); glucagon-like peptide 1 receptor (GLP1R, SEQ ID No:9 and 23); glucagon-like peptide 2 receptor (GLP2R, SEQ ID No:10 and 24); gastric inhibitory polypeptide receptor (GIPR, SEQ ID No:11 and 25); vasoactive intestinal peptide receptor 1 (VIPR1, SEQ ID No:12 and 26); secretin receptor (SCTR, SEQ ID No:13 and 27); and vasoactive intestinal peptide receptor 2 (VIPR2, SEQ ID No:14 and 28) or compounds inhibiting the translation of a polynucleotide sequence selected from the group consisting of SEQ ID Nos.: 1-14 into a polypeptide according to SEQ ID Nos: 15-28, receptively.

The above compounds are used for the preparation of a medicament for prevention and/or treatment, by reducing the level of amyloid-beta protein, of diseases involving cognitive impairment, such as Alzheimer's disease. Antagonists that bind to the polypeptides selected from the group consisting of SEQ ID Nos: 15-28 were shown to reduce the level of amyloid-beta proteins. Therefore, they will be useful in the treatment of diseases such as Alzheimer's disease.

According to a preferred embodiment of the present invention, the antagonists are antagonist of the polypeptide formyl peptide receptor-like 1 (FPRL1) as defined by SEQ ID NO: 15.

Examples of the antagonists according to the present invention are antagonists selected from the group consisting of a peptide comprising the amino acid sequence WRWWWW; chenodeoxycholic acid; cyclosporin (Cs) H; BocPLPLP; Glucagon derivatives; [desHis(1)-[Glu(9)]-glucagon-amide; [desHis(1), Ala(4), Glu(9)] glucagon amide; [desHis(1), D-Ala(4), Glu(9)] glucagon amide; [desHis(1), Leu(4), Glu(9)] glucagon amide; [desHis(1), D-Leu(4), Glu(9)] glucagon amide; NNC 92-1687; BAY 27-9955; alkylidene hydrazide derivatives with alkoxyaryl moieties; [4-hydroxy-3-cyanobenzoic acid (4-isopropylbenzyloxy-3,5-dimethoxymethylene)hydrazide]; 3-cyano-4-hydroxybenzoic acid [1-(2,3,5,6-tetramethylbenzyl)-1H-indol-4-ylmethylene]hydrazide; non-peptide glucagons receptor antagonists; quinoxalines /pyrrolo[1,2 -a]quinoxalines; mercaptobenzimidazoles; 2-pyridyl-3,5-diarylpyrroles; quinoline hydrazones; 4-phenylpyridines; 5-hydroxyalkyl-4-phenylpyridines; triarylimidazole and triarylpyrrole antagonists; an antibody or a fragment thereof; and 2-(-4-Pyridyl)-5-(4-chlorophenyl)-3-(5-bromo-2-propyloxyphenyl)pyrrole.

The translation inhibiting compounds according to the present invention can similar to the above antagonists be used as a novel drug since because the polypeptides of SEQ ID NO: 15-28 increase the level of pathological amyloid beta peptides, inhibiting the translation of these polypeptide will decrease the level of pathological amyloid beta peptides, thus providing a medicament for the treatment of cognitive diseases such as Alzheimer's disease.

As stated above, a preferred polynucleotide sequence according to the present invention is SEQ ID No:1 encoding the polypeptide FPRL1 (SEQ ID No:15).

Examples of such translation inhibitory compound are an antisense RNA, a ribozyme that cleaves the

polyribonucleotide, an antisense oligodeoxynucleotide (ODN), a small interfering RNA (siRNA) that is sufficiently homologous to a portion of the polyribonucleotide such that the siRNA is capable of inhibiting the polyribonucleotide that would otherwise cause the production of the polypeptide, and an antibody reactive to the polypeptide.

Such translation inhibitory compounds can according to the present invention be obtained by a nucleic acid expressing the antisense RNA, a ribozyme that cleaves the polyribonucleotide, an antisense oligodeoxynucleotide (ODN), a siRNA that is sufficiently homologous to a portion of a the polyribonucleotide such that the siRNA is capable of inhibiting the polyribonucleotide that would otherwise cause the production of the polypeptide, or an antibody reactive to the polypeptide.

One type of translation inhibitory agent relates to a nucleic acid that is antisense to a nucleic acid comprising SEQ ID NO: 1-14. For example, an antisense nucleic acid (e.g. DNA) may be introduced into cells in vitro, or administered to a subject in vivo, as gene therapy to inhibit translation of nucleic acids comprising SEQ ID NO: 1-14.

Antisense oligonucleotides preferably comprise a sequence containing from about 17 to about 100 nucleotides and more preferably the antisense oligonucleotides comprise from about 18 to about 30 nucleotides. Antisense nucleic acids may be prepared by expression of all or part of a sequence selected from the group consisting of SEQ ID NO: 1-14, in the opposite orientation. Antisense oligonucleotides can also contain a variety of modifications that confer resistance to nucleolytic degradation such as, for example, modified internucleoside linkages, modified nucleic acid bases and/or modified sugars and the like. The antisense oligonucleotides can also be modified by chemically linking

the oligonucleotide to one or more moieties or conjugates to enhance the activity, cellular distribution, or cellular uptake of the antisense oligonucleotide. Such moieties or conjugates include lipids such as cholesterol, cholic acid, thioether, aliphatic chains, phospholipids, polyamines, polyethylene glycol (PEG), or palmityl moieties.

Another type of translation inhibitory agent relates to a nucleic acid that is able to catalyze cleavage of RNA molecules. The expression "ribozymes" relates to catalytic RNA molecules capable of cleaving other RNA molecules at phosphodiester bonds in a manner specific to the sequence.

The hydrolysis of the target sequence to be cleaved is initiated by the formation of a catalytically active complex consisting of ribozyme and substrate RNA. All ribozymes capable of cleaving phosphodiester bonds in trans, that is to say intramolecularly, are suitable for the purposes of the invention. Apart from ribonuclease P the known naturally occurring ribozymes (hammerhead ribozyme, hairpin ribozyme, hepatitis delta virus ribozyme, Neurospora mitochondrial VS ribozyme, group I and group II introns) are catalysts, which cleave or splice themselves and which act in cis (intramolecularly).

Yet another method of translation inhibition is by small interfering RNAs (siRNAs). siRNAs mediate the post-transcriptional process of gene silencing by double stranded RNA (dsRNA) that is homologous in sequence to the silenced RNA.

Preferably the nucleotide expressing the expression inhibitory agent is included within a vector. Even more preferred, the vector is an adenoviral, retroviral, adeno-associated viral, lenti viral or a sendai viral vector.

A further embodiment of the present invention concerns a method wherein the siRNA comprises a sense strand

of 17-23 nucleotides homologous to a 17-23 nucleotide long nucleotide sequence selected from the group consisting of SEQ ID NO: 1-14 and an antisense strand of 17-23 nucleotides complementary to the sense strand. All nucleotides in the sense and antisense strand base pair, or alternatively there may be mismatches between the sense and antisense strand. Preferably the siRNA further comprises a loop region connecting the sense and the antisense strand.

A self-complementing single stranded siRNA molecule polynucleotide according to the present invention comprises a sense portion and an antisense portion connected by a loop region. Preferably, the second sequence is 4-30 nucleotides long, more preferably 5-15 nucleotides long and most preferably 8 nucleotides long. In a most preferred embodiment the linker sequence is UUGCUAUA (SEQ ID NO: 339).

Self-complementary single stranded siRNAs form hairpin loops and are more stable than ordinary dsRNA. In addition, they are more easily produced from vectors.

Preferably the expression inhibitory agent is an antisense RNA, ribozyme, antisense oligodeoxynucleotide, or siRNA comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338.

The nucleotide sequences are selected according to siRNA designing rules that give an improved reduction of the target sequences compared to nucleotide sequences that do not comply with these siRNA designing rules (See PCT/EP03/04362). A further aspect of the invention relates to a polynucleotide sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338.

Another aspect of the present invention concerns a polynucleotide sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338 for use as a medicament.

Yet another aspect of the present invention relates to the use of a polynucleotide sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338 for the manufacture of a medicament for the treatment of a disease involving cognitive impairment.

Polynucleotides selected from the group consisting of SEQ ID NO: 29-338 can be used in expression inhibitory agents inhibiting the expression of polypeptides of the present invention as described above.

Preferably the polynucleotide is a siRNA.

Another aspect of the invention relates to a vector comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338.

Yet another aspect of the present invention relates to a vector comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338 for use as a medicament.

Furthermore, the present invention relates to the use of a vector comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338 for the manufacture of a medicament for the treatment of a disease involving cognitive impairment.

Preferably the vector encodes a siRNA comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338.

Preferably the vector is an adenoviral, retroviral, adeno-associated viral, lenti viral or a sendai viral vector. In a preferred embodiment of the present invention the disease is Alzheimer's disease.

According another aspect, the present invention provides methods for identifying an antagonist of a polypeptide selected from the group consisting of SEQ ID Nos.: 15-28 or a compound inhibiting the translation of a

polynucleotide sequence selected from the group consisting of SEQ ID Nos.: 1-14 into a polypeptide according to SEQ ID Nos: 15-28, receptively, comprising:

(a) providing a host cell expressing a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID Nos: 15-28, or a fragment, or a derivative thereof;

(b) determining a first activity level of the polypeptide by measuring the level of one or more second messengers of the polypeptide;

(c) exposing the host cell to a compound;

(d) determining a second activity level of the polypeptide by measuring the level of the second messengers after exposing of the compound; and

(e) identifying an antagonist or an inhibiting compound by identifying the compound according to step (c) that provides a difference between the first and the second activity level.

Although we link receptor activation to an increase in second messenger levels a person skilled in the art will understand that receptors that couple to the Gi/o class of G-proteins (such as the FPRL1 receptor) will cause a decrease in cellular cAMP content when activated with an agonist. Exposing the receptor to an antagonist will therefore increase the cAMP levels. Reporter genes that respond to cAMP will therefore follow the cellular cAMP levels. Other second messengers such as Ca2+ will still show an increase when these types of receptors are activated.

The polypeptides of this invention, induce when they are overexpressed or activated the level of secreted amyloid beta 1-42, amyloid beta 1-40, and amyloid beta 1-x, where x ranges from 19-42. Specifically, the amyloid beta peptides 1-

42, 1-40, 1-39, 1-38, 1-37 are often seen in cerebral spinal fluid.

The level of these amyloid beta peptides in Alzheimer patients is increased compared to the levels of these peptides in healthy persons. The amyloid beta peptides 1-42, 1-40, 1-39, 1-38, 1-37 can be found in plaques. Thus, reducing the levels of these amyloid beta peptide is beneficial for patients with cognitive impairment. Therapeutically relevant drug targets may yield an increase in amyloid beta 1-42 levels. The pharmacological inhibition of these targets results in a decrease of amyloid beta levels. The polypeptides of this invention are G-protein coupled receptors (GPCRs) and can be inhibited by small molecules.

All GPCRs share a common architecture of 7 transmembrane domains, an extracellular N-terminus and an intracellular C-terminus. The major signal transduction cascades activated by GPCRs are initiated by the activation of heterotrimeric G-proteins (Wess (1998)), built from three different proteins; the G_α, G_β and G_γ subunits.

The signal transduction cascade starts with the activation of the receptor by an agonist. Transformational changes in the receptor are then translated down to the G-protein. The G-protein dissociates into the G_α subunit and the $G_{\beta\gamma}$ subunit. Both subunits dissociate from the receptor and are both capable of initiating different cellular responses. Best known are the cellular effects that are initiated by the G_α subunit. It is for this reason that G-proteins are categorized by their G_α subunit. The G-proteins are divided into four groups: G_s , $G_{i/o}$, G_q and $G_{12/13}$. Each of these G-proteins is capable of activating an effector protein which results in changes in second messenger levels in the cell. The changes in second messenger level are the triggers

that make the cell respond to the extracellular signal in a specific manner. The activity of a GPCR can be measured by measuring the activity level of the second messenger.

The two most important second messengers in the cell are cAMP and Ca^{2+}. The α-subunit of the G_s class of G-proteins is able to activate adenylyl cyclase, resulting in an increased turnover from ATP to cAMP.

The α-subunit of $G_{i/o}$ G-proteins does exactly the opposite and inhibits adenylyl cyclase activity resulting in a decrease of cellular cAMP levels. Together, these two classes of G-proteins regulate the second messenger cAMP. Ca^{2+} is regulated by the α-subunit of the G_q class of G-proteins.

Through the activation of phospholipase C phosphatidylinositol 4,5-bisphosphate (PIP2) from the cell membrane are hydrolyzed to inositol 1,4,5-trisphosphate and 1,2-diacylglycerol, both these molecules act as second messengers. Inositol 1,4,5-trisphosphate binds specific receptors in the endoplasmatic reticulum, resulting in the opening of Ca^{2+} channels and release of Ca^{2+} in the cytoplasm.

Second messenger activation can be measured by several different techniques, either directly by ELISA or radioactive technologies or indirectly by reporter gene analysis.

A host cell expressing a polypeptide of the present invention can be a cell with endogenous expression of the polypeptide or a cell overexpressing the polypeptide e.g. by transduction. When the endogenous expression of the polypeptide of the present invention is not sufficient for a first activity level of the second measure that can easily be measured, overexpression of the polypeptide can be applied. Overexpression has the advantage that the first activity level of the second messenger is higher than the activity level by endogenous expression.

Preferably the method according to the present invention further comprises contacting the host cell with an agonist for the polypeptide before determining the first activity level. The addition of an agonist further stimulates the polypeptides of the present invention, thereby further increasing the activity level of the second messenger.

As mentioned above, a person skilled in the art will understand that receptors that couple to the $G_{i/o}$ class of G-proteins (such as the FPRL1 receptor) will cause a decrease in cellular cAMP content when activated with an agonist. Exposing the receptor to an antagonist will therefore increase the cAMP levels. Reporter genes that respond to cAMP will therefore follow the cellular cAMP levels. Other second messengers such as Ca2+ will still show an increase when these types of receptors are activated.

Another embodiment relates to the method for identifying an antagonist of a polypeptide selected from the group consisting of SEQ ID Nos.: 15-28 or a compound inhibiting the translation of a polynucleotide sequence selected from the group consisting of SEQ ID Nos.: 1-14 into a polypeptide according to SEQ ID Nos: 15-28, receptively, further comprising

(f) contacting a population of mammalian cells expressing a polypeptide having a amino acid sequences selected from the group consisting of SEQ ID NO: 15-28, or a fragment, or a derivative thereof with the antagonist or the inhibiting compound identified in step (e)

(g) identifying the antagonist or inhibiting compound that reduces the amyloid-beta protein secretion by the cells.

Amyloid-beta precursor protein is processed into several different amyloid beta peptides species. Compounds

are identified that change the APP processing and reduce the level of secreted pathological amyloid beta peptides. Levels of amyloid beta peptides can be measured with specific ELISA's using antibodies specifically recognizing the different amyloid beta peptide species (see e.g. Example 1). Levels of amyloid beta peptides can also be measured by Mass spectrometry analysis (see e.g. Example 7).

A particular embodiment of the present invention relates to a method wherein the polypeptide is FPRL1, as defined by SEQ ID NO: 15.

Another particular embodiment of the present invention relates to a method wherein the polypeptide is GCGR, as defined by SEQ ID NO: 22. Overexpression of FPRL1 or GCGR (example 1) and/or activation of these receptors (example 4) result in increased levels of amyloid beta peptide 1-42, 1-40 and 1-X, where x ranges from 19-42, compared to negative control levels.

A preferred embodiment relates to a method according to the present invention wherein the activity level is determined with a reporter controlled by a promoter, which is responsive to the second messenger.

The reporter is a reporter gene under the regulation of a promoter that responds to the cellular level of second messengers. The reporter gene has a gene product that is easily detected. Reporter genes are easily transferred to host cells by persons skilled in the art. The reporter gene can be stably infected in the host cell.

The reporter gene can be selected from a group comprising: alkaline phosphatase, enhanced green fluorescent protein, destabilized green fluorescent protein, luciferase or b-galactosidase.

Preferably the promoter is a cyclic AMP-responsive promoter, an NF-KB responsive promoter, or a NF-AT responsive

promoter. The cyclic-AMP responsive promoter is responsive for the cyclic-AMP levels in the cell. The NF-AT responsive promoter is sensitive to cytoplasmic Ca2+-levels in the cell. The NF-KB responsive promoter is sensitive for activated NF-kB levels in the cell.

Preferably the reporter is luciferase or b-galactosidase. Luciferase and b-galactosidase are easily available and have a large dynamic range for measuring. In addition, luciferase and b-galactosidase are less expensive which is favorable especially when performing the method of the present invention in a high throughput format.

In another embodiment, the invention relates to a method for identifying an antagonist of a polypeptide selected from the group consisting of SEQ ID Nos.: 15-28 or a compound inhibiting the translation of a polynucleotide sequence selected from the group consisting of SEQ ID Nos.: 1-14 into a polypeptide according to SEQ ID Nos: 15-28, receptively, comprising:

(a) contacting a compound with a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID Nos: 15-28, or a derivative, or a fragment thereof, or with a polynucleotide sequence or a vector comprising a nucleic acid sequence selected from the group consisting of SEQ ID Nos: 1-14;

(b) determining the binding affinity of the compound to the polypeptide or the polynucleotide sequence;

(c) contacting a population of mammalian cells expressing the polypeptide according to SEQ ID. Nos. 15-28 with the compound that exhibits a binding affinity of 10 micromolar or less, and

(d) identifying an antagonist or an inhibiting compound by identifying the compound that provides a decrease in the level of amyloid-beta protein secretion by the mammalian cells.

The binding affinity of the compound with the polypeptide or polynucleotide can be measured by methods known in the art, such as using surface plasmon resonance biosensors (Biacore), by saturation binding analysis with a labeled compound (e.g. Scatchard and Lindmo analysis), by differential UV spectrophotometer, fluorescence polarization assay, Fluorometric Imaging Plate Reader (FLIPR®) system, Fluorescence resonance energy transfer, and Bioluminescence resonance energy transfer.

The binding affinity of compounds can also be expressed in a dissociation constant (Kd) or as IC_{50} or EC50. The IC50 represents the concentration of a compound that is required for 50% inhibition of binding of another ligand to the polypeptide. The EC_{50} represents the concentration required for obtaining 50% of the maximum effect in any assay that measures receptor function.

The dissociation constant, Kd, is a measure of how well a ligand binds to the polypeptide, it is equivalent to the ligand concentration required to saturate exactly half of the binding-sites on the polypeptide. Compounds with a high affinity binding have low Kd, IC_{50} and EC_50 values, i.e. in the range of 100 nM to 1 pM; a moderate to low affinity binding relates to a high Kd, IC_{50} and EC_{50} values, i.e. in the micromolar range.

Changing the APP processing according to the present invention relates to the reduction of the level of amyloid beta peptide 1-x, whereby x ranges from 19-42

and/or the induction of the level of amyloid beta peptide y-42, whereby y ranges from 1-24. The changes in amyloid beta peptide levels can be measured by e.g. an ELISA with specific antibodies as explained in example 1 or by mass spectrometry analysis (example 7).

For high-throughput purposes, libraries of compounds can be used such as peptide libraries (e.g. LOPAPTM, Sigma Aldrich), lipid libraries (BioMol), synthetic compound libraries (e.g. LOPACTM, Sigma Aldrich) or natural compound libraries (Specs, TimTec). Preferably the compounds are low molecular weight compounds. Low molecular weight compounds, i.e. with a molecular weight of 500 Dalton or less, are likely to have good absorption and permeation in biological systems and are consequently more likely to be successful drug candidates than compounds with a molecular weight above 500 Dalton (Lipinski et al. (1997)).

According to another preferred embodiment the compounds are peptides. Many GPCRs have a peptide as an antagonist. Peptides can be excellent drug candidates and there are multiple examples of commercially valuable peptides such as fertility hormones and platelet aggregation inhibitors.

According to another preferred embodiment the compounds are natural compounds. Natural compounds are compounds that have been extracted from e.g. plants or compounds that are synthesized on the basis of a natural occurring molecule. Using natural compounds in screens has the advantage that one screens more diverse molecules. Natural compounds have an enormous variety of different molecules. Synthetic compounds do not exhibit such variety of different molecules.

According to another preferred embodiment the compounds are lipids. Many GPCRs have lipids as antagonists. Using lipids as candidate compounds can increase the chance of finding a specific antagonist for the polypeptides of the present invention.

Another aspect of the present invention concerns a method for diagnosing a pathological condition involving cognitive impairment or a susceptibility to the condition in a subject comprising:

(a) obtaining a sample of the subject's mRNA corresponding to a nucleic acid selected from the group consisting of SEQ ID Nos: 1-14 or a sample of the subject's genomic DNA corresponding to a genomic sequence of a nucleic acid selected from the group consisting of SEQ ID Nos: 1-14;

(b) determining the nucleic acid sequence of the subject's mRNA or genomic DNA;

(c) comparing the nucleic acid sequence of the subject's mRNA or genomic DNA with a nucleic acid selected from the group consisting of SEQ ID Nos: 1-14 or with a genomic sequence encoding a nucleic acid selected from the group consisting of SEQ ID Nos: 1-14 obtained from a database; and

(d) identifying any difference(s) between the nucleic acid sequence of the subject's mRNA or genomic DNA and the nucleic acid selected from the group consisting of SEQ ID Nos: 1-14 or the genomic sequence encoding a nucleic acid selected from the group consisting of SEQ ID Nos: 1-14 obtained from a database.

It is well understood in the art that databases such as GenBank, can be searched to identify genomic sequences that contain regions of identity (exons) to a nucleic acid. Such genomic sequences encode for the nucleic acid.

A further aspect of the present invention relates to method for diagnosing a pathological condition involving cognitive impairment or a susceptibility to the condition in a subject, comprising determining the amount of polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID Nos: 15-28 in a biological sample, and comparing the amount with the amount of the polypeptide in a healthy subject, wherein an increase of the amount of polypeptide compared to the healthy subject is indicative of the presence of the pathological condition. Preferably the pathological condition is Alzheimer's disease.

The term "amyloid beta peptide species" refers to amyloid beta peptides with different composition that are processed from the amyloid beta precursor protein (APP). Examples of the species comprise 1-40, 1-42, y-42, whereby y ranges from 1-24, and 1-x whereby x ranges from 19-42.

The term "expression" comprises both endogenous expression and overexpression by transduction.
The term "compound" comprises organic and inorganic compounds, such as synthetic molecules, peptides, lipids, and natural compounds.

The term "agonist" refers to a ligand that activates the receptor the ligand binds to.

The term "polypeptide" relates to a protein, fractions of a protein, peptides, oligopeptides, or enzymes.

The term "derivatives of a polypeptide" relate to those peptides, oligopeptides, polypeptides, proteins and enzymes that comprise at least about 10 contiguous amino acid residues of the polypeptide and that retain the biological activity of the protein, e.g. polypeptides that have amino acid mutations compared to the amino acid sequence of a naturally-occurring form of the polypeptide.

A derivative may further comprise additional naturally occurring, altered, glycosylated, acylated or non-naturally occurring amino acid residues compared to the amino acid sequence of a naturally occurring form of the polypeptide. It may also contain one or more non-amino acid substituents compared to the amino acid sequence of a naturally occurring form of the polypeptide, for example a reporter molecule or other ligand, covalently or non-covalently bound to the amino acid sequence.

The term "fragment of a polypeptide" relates to peptides, oligopeptides, polypeptides, proteins and enzymes that comprise at least about 5 contiguous amino acid residues, preferably at least 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 contiguous amino acid residues, and exhibit substantially a similar, but not necessarily identical, activity as the complete sequence.

The term "polynucleotide" refers to all nucleic acids, such as DNA and RNA, oligonucleotides. It also includes nucleic acids with modified backbones such as peptide nucleic acid, polysiloxane, and 2'-O-(2-methoxy)ethylphosphorothioate.

The term "derivatives of a polynucleotide" relates to DNA- and RNA- molecules, and oligonucleotides that comprise at least about 10 contiguous nucleic acid

residues of the polynucleotide, e.g. polynucleotides that have nucleic acid mutations compared to the nucleic acid sequence of a naturally-occurring form of the polynucleotide. A derivative may further comprise nucleic acids with modified backbones such as peptide nucleic acid (PNA), polysiloxane, and 2'-O-(2-methoxy)ethyl-phosphorothioate, non-naturally occurring nucleic acid residues, or one or more nuclei acid substituents, such as methyl-, thio-, sulphate, benzoyl-, phenyl-, amino-, propyl-, chloro-, and methanocarbanucleosides, or a reporter molecule to facilitate its detection.

The term "fragment of a polynucleotide" relates to oligonucleotides that comprise at least about 5 contiguous nucleic acid residues, preferably at least 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 contiguous nucleic acid residues, and exhibit substantially a similar, but not necessarily identical, activity as the complete sequence.

FIGURES

Figure 1: APP processing: The membrane anchored amyloid precursor protein (APP) is processed by two pathways: the amyloidogenic and non amyloidogenic pathway. The amyloidogenic pathway generates the pathogenic amyloid beta peptides (Abeta) after cleavage by beta- and gamma-secretase respectively. The numbers depicted are the positions of the amino acids comprising the Abeta sequences.

Figure 2: Evaluation of the APP processing assay: Positive (PS1G384L; PS1L392V and BACE1) and negative (eGFP, LacZ and empty) control viruses were infected in

Hek293APPwt at random MOI. A and B: Transduction was performed respectively with 1 and 0.2 ml of virus and amyloid beta 1-42 levels were determined. Data are represented as relative light units and correlate to pM of amyloid beta 1-42.

Figure 3: Screening results: Hek293 APPwt cells were transduced with a collection of Ad5/GPCRs. The data points from the plate comprising several Ad5/FPRL1 and Ad5/GCGR viruses are depicted. Viruses that scored above the cut-off value stimulate amyloid beta production (1-42) and thus are considered as positives.

Figure 4: Confirmation of the involvement of FPRL1 and GCGR: Hek293 APPwt cells were transduced with Ad5/FPRL1_v1, Ad5/GCGR and with 3 negative control viruses (Ad5/LacZ, Ad5/eGFP and Ad5/luciferase) at different MOIs (2-2500). Resulting amyloid beta 1-42, 1-40 and 1-x peptides were measured with the appropriate ELISA's.

Figure 5: Reporter gene analysis

A: Glucagon dose response curve on HEK293 cells overexpressing the human glucagon receptor. HEK293 cells were transduced with an adenovirus harboring the luciferase gene under the control of a cAMP dependent promoter and a virus harboring the glucagon receptor cDNA. After expressing the receptor, cells were treated with increasing amounts of glucagon. B: Glucagon dose response curve on HEK293 cells expressing the human glucagon receptor. HEK293 cells were transduced with an adenovirus harboring the luciferase gene under the control of a Ca2+ dependent promoter (NFAT elements) and a virus harboring the glucagon receptor cDNA. After expressing the receptor, cells were treated with increasing amounts of glucagon.. C: fMLF dose responds

curve on HEK293 cells expressing the human FPRL1_v1 receptor. HEK293 cells were transduced with an adenovirus harboring the luciferase gene under the control of a cAMP dependent promoter (CRE elements) and a virus harboring the FPRL1 receptor cDNA. After expressing the receptor cells were treated with increasing amounts of fMLF peptide.

Figure 6: Effect of agonists on Abeta 1-42 levels: Hek293 APPwt cells were transduced with Ad5/GCGR (A), Ad5/FPRL1_v1 (B) and Ad5/empty (A and B), and medium containing respectively 5nM glucagon (A), 1mM fMLF (B) or vehicle only was added.

Figure 7: ClustalW protein sequence alignment of GCGR with its closest relatives, being GLP1R and GLP2R. A second ClustalW alignment of the glucagon and the glucagon like peptides is shown.

Figure 8: ClustalW protein sequence alignment of FPRL1 with its closest relatives, being FPR1 and FPRL2.

Figure 9: ClustalW protein sequence alignment of FPRL1_v1 and FPRL1_v2.

Figure 10: Confirmation of the involvement of FPRL1_v2 on Abeta 1-42 production in Hek293 APPwt cells.

Figure 11: Both FPRL1_v1 and FPRL1_v2 increase the production in beta-CTF when overexpressed in HEK293 APP cells.

Figure 12: Both FPRL1_v1 and FPRL1_v2 increase the production in sAPPbeta when overexpressed in HEK293 cells infected with Ad5/APP.

Figure 13: Both FPRL1_v1 and FPRL1_v2 increase the production in Abeta 1-42 when overexpressed in HEK293 cells infected with Ad5/c99.

Figure 14: Both FPRL1_v1 and FPRL1_v2 increase the generation of mature full length APP when overexpressed in HEK293 APP cells.

Figure 15: WKYMVm-mediated signaling of FPRL1_v2 in HEK293 APP cells. HEK293 cells were transduced with Ad5/FPRL1_v2 and Ad/CRE-luc. Cells were treated with increasing amounts of the FPRL1 agonist WKYMVm.

Figure 16: WKYMVm-mediated modulation of Abeta 1-42 levels in HEK293 APP cells infected with FPRL1_v2. HEK293 cells were transduced with Ad5/FPRL1_v2. Cells were treated with increasing amounts of the FPRL1 agonist WKYMVm.

Figure 17: Antagonist-mediated inhibition of WKYMVm-induced Abeta 1-42 levels in HEK293 APP cells infected with Ad5/FPRL1_v2. HEK293 cells were transduced with Ad5/FPRL1_v2. Cells were treated with increasing amounts WKYMVm in absence and presence of two concentrations of a FPRL1 antagonist (WRWWWW; see Bae et al., 2004).

Figure 18: Modulation of Abeta 1-42 by KD of FPRL1 in SH-SY5Y cells.

TABLE 1: GPCRs involved in APP processing:

Accession	Code	Description	SEQ ID NO:	
			DNA	Protein
NM_001462	FPRL1	formyl peptide receptor-like 1	1	15
NM_002030	FPRL2	formyl peptide receptor-like 2	2	16
NM_002029	FPR1	formyl peptide receptor 1	3	17
NM_001506	GPR32	G protein-coupled receptor 32	4	18
NM_004072	CMKLR1	chemokine-like receptor 1	5	19
NM_001736	C5R1	complement component 5 receptor 1 (C5a ligand)	6	20
NM_004778	GPR44	G protein-coupled receptor 44	7	21
NM_000160	GCGR	glucagon receptor	8	22
NM_002062	GLP1R	glucagon-like peptide 1 receptor	9	23
NM_004246	GLP2R	glucagon-like peptide 2 receptor	10	24
NM_000164	GIPR	gastric inhibitory polypeptide receptor	11	25
NM_004624	VIPR1	vasoactive intestinal peptide receptor 1	12	26
NM_002980	SCTR	secretin receptor	13	27
NM_003382	VIPR2	vasoactive intestinal peptide receptor 2	14	28

TABLE2: buffers and solutions used for ELISA

Buffer 42	30mM $NaHCO_3$, 70mM Na_2CO_3, 0.05% NaN_3, pH9.6
Casein buffer	0.1% casein in PBS 1x
EC Buffer	20mM sodium phosphate, 2mM EDTA, 400mM NaCl, 0.2% BSA, 0.05% CHAPS, 0.4% casein, 0.05% NaN_3, pH7
Buffer C	20mM sodium phosphate, 2mM EDTA, 400mM NaCl, 1% BSA, pH7
PBS 10x	80g NaCl + 2g KCl + 11.5g $Na_2HPO_4.7H_2O$ + 2g KH_2PO_4 in 1 l milli Q, pH 7.4
PBST	PBS 1x with 0.05% Tween 20

TABLE 3: Primers used in the quantitative real time PCR analysis for GPCR expression levels

Gene	Primer name	SEQ ID NO:	Primer sequence
FPRL1	FPRL1_Hs_For	340	CCACAAAAAGGGCATGATTAAATC
	FPRL1_Hs_Rev	341	TGAAAGGGAAACCAACAGATGA

TABLE 4: Ct values obtained during quantitative real time PCR: Total human brain, human cerebral cortex or human hippocampus RNA is tested for the presence of FPRL1 RNA via quantitative real time PCR. GAPDH RNA is used to normalize all samples (ΔCt).

Tissue	Ct		ΔCt
	GAPDH	FPRL1	
Total brain	23,53	32,58	9,05
Hippocampus	23,44	32,12	8,68
Cerebral cortex	23,7	32,27	8,57

TABLE 5: Homologues to the GCGR receptor

Gene name	Identical residues (%)	Similar residues (%)
GIPR	50	63
GLP1R	49	64
GLP2R	44	61
VIPR1	41	57
SCTR	39	54
VIPR2	37	53

TABLE 6: Homologues to the FPRL1 receptor

Gene name	Identical residues(%)	Similar residues (%)
FPRL2	71	82
FPR1	68	78
GPR32	40	60
CMKLR1	37	56
C5R1	35	50
GPR44	35	55

5

TABLE 7: Formyl peptide receptor antagonist and agonist

Antagonist	FPR1	FPRL1	FPRL2	Class
Chenodeoxycholic acid	Yes	Yes		Bile acids
Cyclosporin (Cs) H	Yes			
BocPLPLP	Yes			
Agonist	FPR1	FPRL1	FPRL2	Class
Lipoxin A4		Yes		lipoxines
Serum amyloid		Yes		Peptide
MMK-1		Yes		Synthetic peptides
WKYMVm		Yes	Yes	Synthetic peptides
FMLF	Yes	Yes		peptide

TABLE 8: Glucagon receptor antagonist and agonist

Antagonist
Glucagon derivatives such as: [desHis(1)-[Glu(9)]-glucagon-amide [desHis(1), Ala(4), Glu(9)] glucagon amide [desHis(1), D-Ala(4), Glu(9)] glucagon amide [desHis(1), Leu(4), Glu(9)] glucagon amide [desHis(1), D-Leu(4), Glu(9)] glucagon amide
NNC 92-1687
BAY 27-9955
Alkylidene hydrazide derivatives with alkoxyaryl moieties such as: [4-hydroxy-3-cyanobenzoic acid (4-isopropylbenzyloxy-3,5-dimethoxymethylene)hydrazide] 3-cyano-4-hydroxybenzoic acid [1-(2,3,5,6- //

tetramethylbenzyl)-1H-indol-4-ylmethylene]hydrazide
non-peptide glucagons receptor antagonists: quinoxalines /pyrrolo[1,2 -a]quinoxalines mercaptobenzimidazoles 2-pyridyl-3,5-diarylpyrroles q uinoline hydrazones 4-phenylpyridines 5-hydroxyalkyl-4-phenylpyridines
Triarylimidazole and triarylpyrrole antagonist such as: 2-(-4-Pyridyl)-5-(4-chlorophenyl)-3-(5-bromo-2-propyloxyphenyl)pyrrole
Agonist
Glucagon

TABLE 9: Sequences for expression inhibitory agent

Accession number	Name	Sequence	SEQ ID NO:
NM_000164	GIPR	AAATGCGCTGCCGGGATTACC	29
NM_000164	GIPR	AACAGGATTCTAGGCGGAAGC	30
NM_000164	GIPR	AACATCAAGTTCCACACACGC	31
NM_000164	GIPR	AACCCAGAGAAGAATGAGGCC	32
NM_000164	GIPR	AACGGGTCCTTCGATATGTAC	33
NM_000164	GIPR	AAGAATGAGGCCTTTCTGGAC	34
NM_000164	GIPR	AAGCTCGGCTTTGAGATCTTC	35
NM_000164	GIPR	AAGGAGGTGCAGTCGGAGATC	36
NM_000164	GIPR	ACCAAAGGCTCATCTTGGAGC	37
NM_000164	GIPR	ACCATACACAATGTGAGAACC	38
NM_000164	GIPR	ACCTGTTCACGTCTTTCATGC	39
NM_000164	GIPR	ACTTCCGCTACTACCTGCTCC	40
NM_001462	FPRL1	AACCTCTTTGGAAGTGTCTTC	41
NM_001462	FPRL1	AAGACTTAGATGAGATAGCGC	42
NM_001462	FPRL1	AAGGGCATGATTAAATCCAGC	43
NM_001462	FPRL1	AATGCCAGTTCCAGCTTCATC	44

NM_001462	FPRL1	ACACGCACAGTCACCACCATC	45
NM_001462	FPRL1	ACAGTCACCACCATCTGTTAC	46
NM_001462	FPRL1	ACATCGTGGTGGACATCAACC	47
NM_001462	FPRL1	ACCACCATCTGTTACCTGAAC	48
NM_001462	FPRL1	ACCCTTGAGTCATATTGAGGC	49
NM_001462	FPRL1	ACCGCTGCATTTGTGTCCTGC	50
NM_001462	FPRL1	ACGGCCACATTACCATTCCTC	51
NM_001462	FPRL1	ACTGCTGTGGTGGCTTCTTTC	52
NM_001462	FPRL1	ACTTCCGAGAGAGACTGATCC	53
NM_001506	GPR32	AAATGGAATGGCTGTACGCAC	54
NM_001506	GPR32	AACTCTGACAATGAGACTGCC	55
NM_001506	GPR32	AACTGCCTCCTTGTCTTCATC	56
NM_001506	GPR32	AAGATGAACTCTTCCGGATGC	57
NM_001506	GPR32	AATGGCTGTACGCACTGCTAC	58
NM_001506	GPR32	ACATTATAGGGACCATTGGCC	59
NM_001506	GPR32	ACCTTTGTGTTCCTCAGCTAC	60
NM_001506	GPR32	ACGCACTGCTACTTGGCGTTC	61
NM_001506	GPR32	ACGTGGTGCTGTTGGTCCATC	62
NM_001506	GPR32	ACTCTGACAATGAGACTGCCC	63
NM_001506	GPR32	ACTGACTGTGGTTATCCTGTC	64
NM_001506	GPR32	ACTGTCTTCCGTATGGCACGC	65
NM_001506	GPR32	ACTTTGCCAGTAACTGCCTCC	66
NM_001736	C5R1	AAACCCATCTGGTGCCAGAAC	67
NM_001736	C5R1	AACACGCTGCGTGTTCCAGAC	68
NM_001736	C5R1	AACGTGTTGACTGAAGAGTCC	69
NM_001736	C5R1	AACTTGGCGGTAGCCGACTTC	70
NM_001736	C5R1	AAGCGGACCATCAATGCCATC	71
NM_001736	C5R1	AAGCTGGACTCCCTGTGTGTC	72
NM_001736	C5R1	AAGGTGTTGTGTGGCGTGGAC	73
NM_001736	C5R1	AATCCCAGAACTTTGGGAGGC	74
NM_001736	C5R1	AATGATGTCCTTCCTGGAGCC	75
NM_001736	C5R1	AATGCCATCTGGTTCCTCAAC	76
NM_001736	C5R1	AATTAGGCTGAGAGCAGTGGC	77

NM_001736	C5R1	ACAAACAGAAACCCGTGTATC	78
NM_001736	C5R1	ACACTATGGCCCAGAAGACCC	79
NM_001736	C5R1	ACACTTCCTTCTAGGGAGCAC	80
NM_001736	C5R1	ACAGAAGTCCATGGAGTTATC	81
NM_001736	C5R1	ACAGAGGGATCTTGTGTACCC	82
NM_001736	C5R1	ACAGGACATTCTCATCACCAC	83
NM_001736	C5R1	ACATCAACTGCTGCATCAACC	84
NM_001736	C5R1	ACATGTACGCCAGCATCCTGC	85
NM_001736	C5R1	ACCATACCCTCCTTCCTGTAC	86
NM_001736	C5R1	ACCATCAATGCCATCTGGTTC	87
NM_001736	C5R1	ACCTTAGCTAACTAACTCTCC	88
NM_001736	C5R1	ACGCTGCGTGTTCCAGACATC	89
NM_001736	C5R1	ACGTCCATTGTACAGCATCAC	90
NM_001736	C5R1	ACTAACTCTCCTCCATGTTGC	91
NM_001736	C5R1	ACTACAGCCACGACAAACGGC	92
NM_001736	C5R1	ACTTCCTTCTAGGGAGCACCC	93
NM_002030	FPRL2	AAAGACTGATTCGCTCTTTGC	94
NM_002030	FPRL2	AACACCATCTGTTACCTGAAC	95
NM_002030	FPRL2	AACCCAACAAGCTCCTTGGCC	96
NM_002030	FPRL2	AACCTGGCCCTAGCTGACTTC	97
NM_002030	FPRL2	AACCTGTTTGTCAGTGTCTAC	98
NM_002030	FPRL2	AACGTGTTCATTACCATGGCC	99
NM_002030	FPRL2	AAGACTGATTCGCTCTTTGCC	100
NM_002030	FPRL2	AAGAGGGTGATGACGGGACTC	101
NM_002030	FPRL2	AAGGTCTTTCTGATCCTCCAC	102
NM_002030	FPRL2	ACACACCACTTCTGCTTCACC	103
NM_002030	FPRL2	ACACCACTTCTGCTTCACCTC	104
NM_002030	FPRL2	ACACCATCTGTTACCTGAACC	105
NM_002030	FPRL2	ACACGCACAGTCAACACCATC	106
NM_002030	FPRL2	ACAGCTGCCTCAACCCAATTC	107
NM_002030	FPRL2	ACAGTCAACACCATCTGTTAC	108
NM_002030	FPRL2	ACAGTCTGCTATGGGATCATC	109
NM_002030	FPRL2	ACATGATTAAATCCAGCCGTC	110

NM_002030	FPRL2	ACCACATGATTAAATCCAGCC	111
NM_002030	FPRL2	ACCATCATTGCTCTGGACCGC	112
NM_002030	FPRL2	ACCGCTGTATTTGTGTCCTGC	113
NM_002030	FPRL2	ACCTGATCACCATCATTGCTC	114
NM_002030	FPRL2	ACCTGTTTGTCAGTGTCTACC	115
NM_002030	FPRL2	ACGGTGCCTATGTCCATCATC	116
NM_002030	FPRL2	ACTGATTCGCTCTTTGCCCAC	117
NM_002030	FPRL2	ACTGCTGTAGAGAGGTTGAAC	118
NM_002030	FPRL2	ACTTCTCTTTCAGTGCCATCC	119
NM_002062	GLP1R	AAATGCAGACTTGCCAAGTCC	120
NM_002062	GLP1R	AAATGGCGAGAATACCGACGC	121
NM_002062	GLP1R	AACCGGACCTTCGATGAATAC	122
NM_002062	GLP1R	AACCTCAGCCAAACACAGAGC	123
NM_002062	GLP1R	AACCTGTTTGCATCCTTCATC	124
NM_002062	GLP1R	AAGAGACTCTCTTAGGGAAAC	125
NM_002062	GLP1R	AAGAGAGACATTGCCTCCACC	126
NM_002062	GLP1R	AAGCAGCCTCCTAATTTGATC	127
NM_002062	GLP1R	AAGCTGTTTACAGAGCTCTCC	128
NM_002062	GLP1R	AAGGGAAGCTGTTTGTGTGTC	129
NM_002062	GLP1R	AAGTCCACGCTGACACTCATC	130
NM_002062	GLP1R	AAGTGGATGTATAGCACAGCC	131
NM_002062	GLP1R	AATCTCATGTGCAAGACAGAC	132
NM_002062	GLP1R	AATGGCGAGAATACCGACGCC	133
NM_002062	GLP1R	AATGGGCAATTCTGACTTCTC	134
NM_002062	GLP1R	AATTTCGGAAGAGCTGGGAGC	135
NM_002062	GLP1R	ACAATGGGCAATTCTGACTTC	136
NM_002062	GLP1R	ACACACACACATACATCCTGC	137
NM_002062	GLP1R	ACACACATACATCCTGCTTTC	138
NM_002062	GLP1R	ACACATACATCCTGCTTTCCC	139
NM_002062	GLP1R	ACACGTTAGGAATGTCCAGAC	140
NM_002062	GLP1R	ACAGAGCTCTCCTTCACCTCC	141
NM_002062	GLP1R	ACAGCAGCACTGCAGATAGCC	142
NM_002062	GLP1R	ACATACATCCTGCTTTCCCTC	143

NM_002062	GLP1R	ACATGGCTATCCTAGAGAGGC	144
NM_002062	GLP1R	ACCAGGAACTCCAACATGAAC	145
NM_002062	GLP1R	ACCTGTTTGCATCCTTCATCC	146
NM_002062	GLP1R	ACCTTCGATGAATACGCCTGC	147
NM_002062	GLP1R	ACGCACTCTCCTTCTCTGCTC	148
NM_002062	GLP1R	ACTACTGGCTCATTATCCGGC	149
NM_002062	GLP1R	ACTCATGAGGTCATCTTTGCC	150
NM_002062	GLP1R	ACTCCAACATGAACTACTGGC	151
NM_002062	GLP1R	ACTGCACCAGGAACTACATCC	152
NM_002062	GLP1R	ACTGGCTCATTATCCGGCTGC	153
NM_002062	GLP1R	ACTTGCCAAGTCCACGCTGAC	154
NM_002062	GLP1R	ACTTTATCTGTGACCACACGC	155
NM_002980	SCTR	AAAGTCATGTACACCGTGGGC	156
NM_002980	SCTR	AACGAGAAGCGGCACTCCTAC	157
NM_002980	SCTR	AACGCATCCATCTGGTGGATC	158
NM_002980	SCTR	AACTAGCCCTTGGCTCATTCC	159
NM_002980	SCTR	AAGAAGTGGCAGCAATGGCAC	160
NM_002980	SCTR	AAGACCAGTGCCTGCAGGAAC	161
NM_002980	SCTR	AAGAGCAAGACCAGTGCCTGC	162
NM_002980	SCTR	AAGCTGAAAGTCATGTACACC	163
NM_002980	SCTR	AAGCTGGTCATGGTGCTGTTC	164
NM_002980	SCTR	AAGGCCTCTACCTTCACACAC	165
NM_002980	SCTR	AAGTCAGCCATTATAAGCGCC	166
NM_002980	SCTR	AAGTGGCAGCAATGGCACCTC	167
NM_002980	SCTR	AATGGCACCTCCGTGAGTTCC	168
NM_002980	SCTR	AATGGTTCCTTGTTCCGAAAC	169
NM_002980	SCTR	AATGTGAACGACTCTTCCAAC	170
NM_002980	SCTR	ACAAGAGGAAATGAAGTCAGC	171
NM_002980	SCTR	ACACTCCTCGCCATCTCCTTC	172
NM_002980	SCTR	ACAGGATGGCTGGTCAGAAAC	173
NM_002980	SCTR	ACATCGTCTTCGCCTTCTCCC	174
NM_002980	SCTR	ACCTGCAGGACCAGCATCATC	175
NM_002980	SCTR	ACCTGTTCGTGTCCTTCATCC	176

NM_002980	SCTR	ACGACTCTTCCAACGAGAAGC	177
NM_002980	SCTR	ACTCTTCCAACGAGAAGCGGC	178
NM_002980	SCTR	ACTGCACTCGCAACTACATCC	179
NM_002980	SCTR	ACTTCATCAAGGACGCCGTGC	180
NM_003382	VIPR2	AAACACAAAGCCTGCAGTGGC	181
NM_003382	VIPR2	AAACTGTTTCAAGCCCTCCTC	182
NM_003382	VIPR2	AAAGCAGCAGTGTCCAGAGAC	183
NM_003382	VIPR2	AAAGCCTGATCTCACATCTGC	184
NM_003382	VIPR2	AAATCAACAGGAGGGCAGCCC	185
NM_003382	VIPR2	AACCTGTTCCTGTCCTTCATC	186
NM_003382	VIPR2	AACTGTTTCAAGCCCTCCTCC	187
NM_003382	VIPR2	AAGCAGCAGTGTCCAGAGACC	188
NM_003382	VIPR2	AAGCAGGACCCAGTGGTCAAC	189
NM_003382	VIPR2	AAGCTGGTTGTCCACTAAACC	190
NM_003382	VIPR2	AAGGACGACGTTCTCTACTCC	191
NM_003382	VIPR2	AAGGAGGAAATGTGGAAACGC	192
NM_003382	VIPR2	AAGGCCATTTATACCCTGGGC	193
NM_003382	VIPR2	AAGTCCACGCTCCTGCTTATC	194
NM_003382	VIPR2	ACAAACGACCACAGTGTGCCC	195
NM_003382	VIPR2	ACAAGCTCATCCCTGGACTTC	196
NM_003382	VIPR2	ACACATCCTGTCAGTGTCACC	197
NM_003382	VIPR2	ACAGGAAGCATAATTCTGTGC	198
NM_003382	VIPR2	ACAGGGTTTCACCATGTTAGC	199
NM_003382	VIPR2	ACAGTGTCTCTCTGATGTCTC	200
NM_003382	VIPR2	ACATCCACCTGAACCTGTTCC	201
NM_003382	VIPR2	ACATGGTGTTTGCCGTGTTTC	202
NM_003382	VIPR2	ACCAGTCTCAGTACAAGAGGC	203
NM_003382	VIPR2	ACCATGTTAGCCAGGATGGTC	204
NM_003382	VIPR2	ACCCAGTGGTCAACAGGTGTC	205
NM_003382	VIPR2	ACCGCACATGTGCCACTGTTC	206
NM_003382	VIPR2	ACCGGTTGCTGGGATACAAAC	207
NM_003382	VIPR2	ACCTCTCCACACAGGTGTTCC	208
NM_003382	VIPR2	ACCTGTTCCTGTCCTTCATCC	209

NM_003382	VIPR2	ACGGATGGTCAGAGACGTTCC	210
NM_003382	VIPR2	ACGTTAGGACCAGGAGAAATC	211
NM_003382	VIPR2	ACTCCGTCAAGCTGGTTGTCC	212
NM_003382	VIPR2	ACTGCACCAGGAATTACATCC	213
NM_003382	VIPR2	ACTGTTTCAAGCCCTCCTCCC	214
NM_004072	CMKLR1	AACATGGTCTGGTTCCTCAAC	215
NM_004072	CMKLR1	AACCTCCTAGAGCTCCACCAC	216
NM_004072	CMKLR1	AACCTGGCAGTGGCAGATTTC	217
NM_004072	CMKLR1	AACTTCCTTCTCATCCACAAC	218
NM_004072	CMKLR1	AAGAACCTCTTTAGCATCCAC	219
NM_004072	CMKLR1	AAGAAGACAGTGAACATGGTC	220
NM_004072	CMKLR1	AAGAAGTTCAAGGTGGCCCTC	221
NM_004072	CMKLR1	AAGATCAGCAACTTCCTTCTC	222
NM_004072	CMKLR1	AAGTGAAGATACAGGCCACTC	223
NM_004072	CMKLR1	AAGTTCAAGGTGGCCCTCTTC	224
NM_004072	CMKLR1	AATCCATATCACCTATGCCGC	225
NM_004072	CMKLR1	AATTTATGCTTCTTGGGAGGC	226
NM_004072	CMKLR1	ACAACTTCAGCCTGTCCACAC	227
NM_004072	CMKLR1	ACACACTCAACCTCCTAGAGC	228
NM_004072	CMKLR1	ACACTCAACCTCCTAGAGCTC	229
NM_004072	CMKLR1	ACAGCCATGTGCAAGATCAGC	230
NM_004072	CMKLR1	ACAGCTTGCTACCTCACCATC	231
NM_004072	CMKLR1	ACAGTGAACATGGTCTGGTTC	232
NM_004072	CMKLR1	ACATGCTGTGTTCCATACAGC	233
NM_004072	CMKLR1	ACATGGTCTGGTTCCTCAACC	234
NM_004072	CMKLR1	ACATGGTGGTGACTGTCACCC	235
NM_004072	CMKLR1	ACATGTTCACCAGCGTCTTCC	236
NM_004072	CMKLR1	ACCATCATCAGCTCTGACCGC	237
NM_004072	CMKLR1	ACCTATGCCGCCATGGACTAC	238
NM_004072	CMKLR1	ACCTCACCATCGTGTGCAAAC	239
NM_004072	CMKLR1	ACCTGGCAGTGGCAGATTTCC	240
NM_004072	CMKLR1	ACCTTCTTCCTCTGCTGGTGC	241
NM_004072	CMKLR1	ACTCTCTCAACCCAGGGACAC	242

NM_004072	CMKLR1	ACTGCCCTTGCCATTGCCAAC	243
NM_004246	GLP2R	AAACAGGCATGTCTGAGAGAC	244
NM_004246	GLP2R	AAACGACTCGGAAGTGGGCTC	245
NM_004246	GLP2R	AAACTCCACTGCACGCGCAAC	246
NM_004246	GLP2R	AAATGTCTCTGTACCCTGCCC	247
NM_004246	GLP2R	AACCTTGCAGCTGATGTACAC	248
NM_004246	GLP2R	AACGGGACATTTGATCAGTAC	249
NM_004246	GLP2R	AAGCAAGTTACAGGATCCCTC	250
NM_004246	GLP2R	AAGCTCTCGGAAGGAGATGGC	251
NM_004246	GLP2R	AAGCTGCAGCCCTCACTTAAC	252
NM_004246	GLP2R	AAGGACGTCGTCTTCTACAAC	253
NM_004246	GLP2R	AAGGAGATGGCGCTGAGAAGC	254
NM_004246	GLP2R	AAGGCTGAGCTGCGGAAATAC	255
NM_004246	GLP2R	AATACTGGGTCCGCTTCTTGC	256
NM_004246	GLP2R	AATCAACACTGGTCCTCATTC	257
NM_004246	GLP2R	AATGAGAATGGGTGGATGTCC	258
NM_004246	GLP2R	ACAACTCTTACTCCAAGAGGC	259
NM_004246	GLP2R	ACCAGTCCTCTCTCCTTCCAC	260
NM_004246	GLP2R	ACCCATGATGCTCTGTGTAAC	261
NM_004246	GLP2R	ACCCTGCCCTTCATACTTACC	262
NM_004246	GLP2R	ACCTTGCAGCTGATGTACACC	263
NM_004246	GLP2R	ACGGATATTTGGCAGGATGAC	264
NM_004246	GLP2R	ACGTGGACCGTTATGCCTTGC	265
NM_004246	GLP2R	ACTCCGAATGCTCCGAGAACC	266
NM_004246	GLP2R	ACTCCTTCTCTCTTATCTCCC	267
NM_004246	GLP2R	ACTCGGAAGTGGGCTCAGTAC	268
NM_004246	GLP2R	ACTCTGGTCCTGCTGGTTTCC	269
NM_004246	GLP2R	ACTTGGCAGACGATAGAGAAC	270
NM_004624	VIPR1	AAAGACTTGGCCCTCTTCGAC	271
NM_004624	VIPR1	AAAGCAGATACCTCACCCTGC	272
NM_004624	VIPR1	AACAGGAATCAAGAGCTGCCC	273
NM_004624	VIPR1	AACCCAAGGACTGAGGGACTC	274
NM_004624	VIPR1	AACTACATCCACATGCACCTC	275

NM_004624	VIPR1	AACTCAGTCATTAGACTCCTC	276
NM_004624	VIPR1	AACTCCTCACTGTGGTGGATC	277
NM_004624	VIPR1	AAGACCGGCTACACCATTGGC	278
NM_004624	VIPR1	AAGAGTGACAGCAGTCCATAC	279
NM_004624	VIPR1	AAGATGGTCTTTGAGCTCGTC	280
NM_004624	VIPR1	AAGATGTGGGACAACCTCACC	281
NM_004624	VIPR1	AAGCCTGAAGTGAAGATGGTC	282
NM_004624	VIPR1	AAGGTCACCAGCACCAACACC	283
NM_004624	VIPR1	AAGTCTCCCTGGTCTGACCAC	284
NM_004624	VIPR1	AAGTGAAGATGGTCTTTGAGC	285
NM_004624	VIPR1	AAGTGAGAGAGATGGGAGCTC	286
NM_004624	VIPR1	AATGAGAAGGCAGCCACCAGC	287
NM_004624	VIPR1	AATGAGACAATAGGCTGCAGC	288
NM_004624	VIPR1	AATGTAAGCCGCAGCTGCACC	289
NM_004624	VIPR1	ACACCTATCTTAGTGGTTCCC	290
NM_004624	VIPR1	ACACCTCTGCCAGAAGATCCC	291
NM_004624	VIPR1	ACACTCCTAGAGAACGCAGCC	292
NM_004624	VIPR1	ACAGAAAGCAGATACCTCACC	293
NM_004624	VIPR1	ACAGCAGTCCATACTCAAGGC	294
NM_004624	VIPR1	ACAGCTATCCTGAGCCTGTTC	295
NM_004624	VIPR1	ACATCATGTTCGCCTTCTTTC	296
NM_004624	VIPR1	ACATTCACCATGGTGTGGACC	297
NM_004624	VIPR1	ACCGGCTACACCATTGGCTAC	298
NM_004624	VIPR1	ACCGGTGGATCCTCAAACAAC	299
NM_004624	VIPR1	ACCTCACCCTGCTACACATAC	300
NM_004624	VIPR1	ACCTCCATCTTGGTAAACTTC	301
NM_004624	VIPR1	ACCTCTTCATATCCTTCATCC	302
NM_004624	VIPR1	ACGCAGGTTTCCATGCTGACC	303
NM_004624	VIPR1	ACTAGGCTCAGAGATGTGCAC	304
NM_004624	VIPR1	ACTCAGCTTCCTACCCACACC	305
NM_004624	VIPR1	ACTCAGTCATTAGACTCCTCC	306
NM_004624	VIPR1	ACTGAAGATGCAGCTCACTAC	307
NM_004624	VIPR1	ACTGAGGGACTCTGAAGCCTC	308

NM_004624	VIPR1	ACTGCAACAGGCTTGTGCAAC	309
NM_004624	VIPR1	ACTGCACGCGGAACTACATCC	310
NM_004624	VIPR1	ACTTTCATCCTGACTCTGCCC	311
NM_004778	GPR44	AAACTCTTGAGATCTTGGTCC	312
NM_004778	GPR44	AAACTGCACTCCTCCATCTTC	313
NM_004778	GPR44	AAAGGGAACAGTGAGGTGCCC	314
NM_004778	GPR44	AAAGTATCACCAGGGTGCCGC	315
NM_004778	GPR44	AACAGTGAGTTAAAGCAGTGC	316
NM_004778	GPR44	AACATGTTCGCCAGCGGCTTC	317
NM_004778	GPR44	AACCCTAGGCATCACATGCTC	318
NM_004778	GPR44	AACTCGTAATAGACTTCCCAC	319
NM_004778	GPR44	AACTCTAAGACTACAGCACAC	320
NM_004778	GPR44	AACTTGCACCTCTGACCTATC	321
NM_004778	GPR44	AAGGTTTGAGAAGCACTGTTC	322
NM_004778	GPR44	AAGTGCTTCCAAGGCAGAAGC	323
NM_004778	GPR44	AAGTTGAATGGGCACAGCAAC	324
NM_004778	GPR44	AATCCCAAGATCTGTGCAGCC	325
NM_004778	GPR44	AATGCTTACTGCGCTAGACGC	326
NM_004778	GPR44	ACAATGTGCTGCTCCTGAACC	327
NM_004778	GPR44	ACAGGGTCTGCACTCTAACCC	328
NM_004778	GPR44	ACCACCTTCTGCAAACTGCAC	329
NM_004778	GPR44	ACCAGCATCCGCTACATCGAC	330
NM_004778	GPR44	ACCAGCCTGGCCTTCTTCAAC	331
NM_004778	GPR44	ACCTATCACTTCCACTGCACC	332
NM_004778	GPR44	ACCTTCTGCAAACTGCACTCC	333
NM_004778	GPR44	ACCTTGATGTGCCTGTGAATC	334
NM_004778	GPR44	ACGGTGCCCTATTTCGTGTTC	335
NM_004778	GPR44	ACTCACACGCGAAAGTATCAC	336
NM_004778	GPR44	ACTGCGCTAGACGCTTCATCC	337
NM_004778	GPR44	ACTGCTGTGTTTGAGCTCTGC	338

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EXAMPLES

EXAMPLE 1: GPCRs modulate amyloid beta 1-42 levels

Constucts used in these studies

BACE1: The cDNA encoding transcript "a" of Homo sapiens beta-site APP-cleaving enzyme (BACE) was obtained by PCR on a human heart cDNA library with following primers:

forward primer: GCGAAGCTTGCCACCAGCACCACCCAGACT (Seq ID no 342)

reverse primer: GGGGGATCCATTTGGTGGGTGGGGAGGGTC. (Seq ID no 343)

The PCR amplification yielded a 1740 bp DNA. The aforementioned primers were designed in such a way that the PCR product could be inserted into the pIPspAdapt6 vector by HindIII-BamHI cloning. The full-length sequence of this cDNA corresponded to the coding sequence of NM_012104.

PS1L392V (PS1_v3): The cDNA encoding Homo sapiens presenilin 1 (PSEN1) was isolated from human placenta cDNA library (constructed in pIPspAdapt6 using a SalI-NotI cloning strategy) by a classical filter colony hybridisation strategy. A bacterial colony at a position corresponding to that of a positive signal spot on the filter was picked and used for plasmid preparation. Sequence verification confirmed that the insert corresponded to the coding sequence of NM_000021.

Next, a clinical mutant was introduced to yield a Leu-Val exchange at position 392 by means of an overlap PCR on two pre-generated PCR fragments. Those two fragments were formed by means of following primers:

Fragment a, forward primer: GGTGGGAGGTCTATATAAGC (Seq ID no 344)

Fragment a, reverse primer (inserts reverse mutation):

CTGTTGCTGAGGCCTTACCAACCACAACACTGTA GAAAATGAAATC. (Seq ID no 345)

Fragment b: forward primer (inserts forward mutation):

GATTTCATTTTCTACAGTGTTGTGGTTGGTAA GGCCTCAGCAACAG
(Seq ID no 346)
Fragment b, reverse primer:
GGACAAACCACAACTAGAATGC
(Seq ID no 347)

Both PCR fragments were assembled with following primers:

forward primer: GGTGGGAGGTCTATATAAGC
(Seq ID no 348)
reverse primer: GGACAAACCACAACTAGAATGC
(Seq ID no 349)

A HindIII-BamHI product of 1592 bp was inserted into the pIPspAdapt6 vector. Sequence verification confirmed that the insert still corresponded to the coding sequence of NM_000021 and that the desired mutation was introduced.

PS1G384A (PS1_v5): This clinical mutant was introduced to yield a Gly-Ala exchange at position 384 by means of an overlap PCR on two pre-generated PCR fragments. Those two fragments were formed by means of following primers:

Fragment a, forward primer: GGTGGGAGGTCTATATAAGC (Seq ID no 350)

Fragment a, reverse primer (inserts reverse mutation):
CACTGTAGAAAATGAAATCTGCCAATCCAAGCTTTA
CTCCCCTTTCCTCTGGG (Seq ID no 351)
Fragment b: forward primer (inserts forward mutation):

CCCAGAGGAAAGGGGAGTAAAGCTTGGATTGGCAGATTTCATTTTCTAC AGTG (Seq ID no 352)

Fragment b, reverse primer:

GGACAAACCACAACTAGAATGC (Seq ID no 353)

Both PCR fragments were assembled with following primers:

forward primer: GGTGGGAGGTCTATATAAGC (Seq ID no 354)

reverse primer: GGACAAACCACAACTAGAATGC (Seq ID no 355)

An EclIXI-BamHI product of 1612 bp was inserted into the pIPspAdapt6 vector. Sequence verification confirmed that the insert corresponded to the coding sequence of NM_000021 and that the desired mutation was introduced.

dE1/dE2A adenoviruses were generated from these adapter plasmids by co-transfection of the helper plasmid pWEAd5AflII-rITR.dE2A in PER.C6/E2A packaging cells, as described (WO99/64582). LacZ: Described as pIPspAdApt6-lacZ in WO02070744. Empty: is generated from pIPspAdApt 6. The generation of this virus is described in WO02070744. EGFP: Described as pIPspAdApt6-EGFP in WO02070744.

Luciferase (A010800-luc_v17): The luciferase reporter was recloned into pIPspAdApt 6 (WO 02070744) from the pCLIP-luciferase construct described in WO 02070744. Viruses were generated as described (WO 02070744).

Luciferase reporter constructs: pIPspAdapt6 (WO 02070744) was digested with SalI and NotI restriction

enzymes and purified over gel. The reporter cassettes, containing multiple copies of cAMP; NFkB; and NFAT-responsive elements, respectively, in front of a minimal promotor driving expression of luciferase, were digested with SalI and NotI restriction enzymes, purified over gel, and ligated into the linearized adapter vector. The resulting vectors were further processed by AvrII - SalI digestion to remove the CMV-promotor region, whereafter the vector was blunted and relegated, resulting in the final reporter constructs Adenoviruses were generated according to 99-64582.

eGFP knock-down (A150100-eGFP_v6) Target sequence: 5'-GAACGGCATCAAGGTGAAC. ((Seq ID no 356) Cloned using Sap1-sites into vector and virus generated as described in WO03/020931.

APP: The cDNA encoding Homo sapiens APP770 was isolated from a human placenta cDNA library (see WO 02070744) by classical filter colony hybridisation. A bacterial colony at a position corresponding to that of a positive signal spot on the filter after hybridisation was picked and used for plasmid preparation. Full-length sequence verification confirmed that the insert corresponded to the coding sequence of NM_000484. The APP770 isoform was transformed into the APP695 isoform according to standard molecular biology procedures. Viruses were generated as described (WO 02070744).

APPsw: The APP770 isoform, as described above, was used to introduce the Swedish clinical mutation (a Lys670Asn and a Met671Leu exchange) by means of an overlap PCR on two pre-generated PCR fragments. The resulting PCR fragment was inserted into the pIPspAdapt6 vector. The APP770 isoform was transformed into the APP695 isoform according to standard molecular biology

procedures. Viruses were generated as described (WO 02070744).

C99: This construct contains the APP signal peptide with the 99 C-terminal amino acids of human APP. The amplification of the signal peptide was performed by PCR and the resulting PCR fragment was treated with restriction enzymes AgeI and EcoRI. The vector holding the full length APP sequence was digested with AgeI and EcoRI to generate the C99 fragment. The signaling peptide fragment was then ligated in frame into the C99 fragment of APP. Viruses were generated as described (WO 02070744)

To identify novel drug targets that change the APP processing, a stable cell line overexpressing APP, Hek293 APPwt, was transduced with adenoviral cDNA libraries and the resulting amyloid beta 1-42 levels were detected via ELISA. This stable cell line was created after transfection of Hek293 cells with the APP770wt cDNA cloned in pcDNA3.1 and selection with G418 during 3 weeks. At this time point colonies were picked and stable clones were expanded and tested for their secreted amyloid beta peptide levels.

The assay was performed as follows. Cells seeded in collagen-coated plates at a cell density of 15000 cells/well (384 well plate) in DMEM 10%FBS, were infected 24 h later with 1 ml or 0.2 ml of adenovirus (corresponding to an average multiplicity of infection (MOI) of 120 and 24 respectively). The following day, the virus was washed away and DMEM containing 25 mM Hepes and 10%FBS was added to the cells. Amyloid beta peptides were allowed to accumulate during 24h. The ELISA plate was prepared by coating the capture antibody (JRF/cAbeta42/26) (obtained from M Mercken, Jóhnson and

Johnson Pharmaceutical Research and Development, B-2340 Beerse, Belgium) overnight in buffer 42 (Table 2) at a concentration of 2,5 mg/ml. The excess capture antibody was washed away the next morning with PBS and the ELISA plate was blocked overnight with casein buffer (Table 2) at 4°C. Upon removal of the blocking buffer, 30 ml of the sample was transferred to the ELISA plate and incubated overnight at 4°C. After extensive washing with PBS-Tween20 and PBS, 30 ml of the horse reddish peroxidase (HRP) labeled detection antibody (Peroxidase Labeling Kit, Roche), JRF/AbetaN/25-HRP (obtained from M Mercken, Johnson and Johnson Pharmaceutical Research and Development, B-2340 Beerse, Belgium) was diluted 1/5000 in buffer C (Table 2) and added to the wells for another 2h. Following the removal of excess detection antibody by a wash with PBS-Tween20 and PBS, HRP activity was detected via addition of luminol substrate (Roche), which is converted into a chemiluminescent signal by the HRP enzyme.

In order to validate the assay, the effect of adenoviral overexpression of two clinical PS1 mutants and BACE on amyloid beta 1-42 production was evaluated in the Hek293 APPwt cells. As is shown in Figure 2, all constructs induced amyloid beta 1-42 levels as expected.

An adenoviral cDNA library containing almost all GPCRs was constructed as follows. DNA fragments covering the full coding region of the GPCRs, were amplified by PCR from a pooled placental and fetal liver cDNA library (InvitroGen). All fragments were cloned into our proprietary adenoviral vector (see US 6,340,595) and subsequently adenoviruses were made harboring the corresponding cDNAs. During the screening of the adenoviral GPCR library in the Hek293 APPwt cells, FPRL1

and GCGR were identified as modulators of APP processing. (see Figure 3). 3 adenoviruses harboring clones of GCGR scored above the cut-off value, while 2 adenoviruses harboring different variants for FPRL, FPRL1_v1 and FPRL1_v2 (see Figure 9), scored positive. These results indicate that overexpression of FPRL1 and GCGR lead to increased levels of amyloid beta 1-42 peptides in the conditioned medium of Hek293 APPwt cells, showing that both GPCRs modulate APP processing.

The stimulatory effect of FPRL1 and GCGR was confirmed upon re-screening of the viruses with a known titer (viral particles/ml), as determined by quantitative real time PCR. For this, cells were infected with FPRL1 and GCGR viruses at MOIs ranging from 2 to 1250 and the experiment was performed as described above. Amyloid beta 1-42 levels were significantly higher for Ad5/FPRL1_v1 and Ad5/FPRL1_v2, and Ad5/GCGR clones at MOI 1250 compared to the negative controls (Figures 4A and 10). In addition, the effect of FPRL1_v1 and GCGR on amyloid beta 1-40 and 1-x levels were checked under similar conditions as above. The respective ELISA's were performed as described above, except that the following antibodies were used: for the amyloid beta 1-40 ELISA, the capture and detection antibody were respectively JRF/cAbeta40/10 and JRF/AbetaN/25-HRP (obtained from M Mercken, Johnson and Johnson Pharmaceutical Research and Development, B-2340 Beerse, Belgium), while for the amyloid beta 1-x ELISA (x ranges from 19-42) the capture and detection antibodies were JRF/AbetaN/25 and 4G8-HRP, respectively (obtained respectively from M Mercken, Johnson and Johnson Pharmaceutical Research and Development, B-2340 Beerse, Belgium and from Signet, USA).

The amyloid beta 1-x ELISA was used for the detection of amyloid peptides with a variable C-terminus (amyloid beta 1-37; 1-38; 1-39; 1-40; 1-42). The results of these experiments clearly showed an increase of amyloid beta 1-40 and 1-x species upon transduction of FPRL1_v1 and GCGR (Figure 4B and 4C).

These are surprising results according to what is known about FPRL1 and its relation to amyloid peptides. Classic studies suggested that the N-formyl group was a crucial determinant of ligand binding and because bacterial and mitochondrial proteins are the only sources in nature, it was widely thought that these receptors evolved to mediate trafficking of phagocytes to sites of bacterial invasion or tissue damage. However, over the past five years, data from several groups have indicated that these receptors might act in a more complex manner, since a large number of non-formylated peptide ligands have now been identified. FPRL1 is known as a GPCR that has both endogenous peptide and lipid (lipoxin A4) ligands. At least three host-derived polypeptides are identified as ligands for this receptor, which are all associated with amyloidogenic diseases: serum amyloid A, prion protein fragment106-126 and amyloid beta 1-42.

The relevance of FPRL1 to Alzheimer's disease is in its relation to the inflammatory aspects of the disease and is underscored by FPRL1 being a chemotactic receptor for amyloid beta 1-42, which induces monocyte migration and activation. In brain tissue of ALZHEIMER'S DISEASE patients, mononuclear phagocytes that surround or infiltrate the plaques express high levels of FPRL1. In addition, FPRL1 can promote the cellular uptake of amyloid beta 1-42 by rapid internalization into the

cytoplasmic compartment in the form of amyloid beta 1-42-FPRL1 complexes. Moreover, amyloid fibrils and aggregates are accumulated in macrophages in an FPRL1-mediated fashion. Hence, following roles in the mechanisms of amyloid beta 1-42 amyloid aggregation and degradation are suggested: intracellular fibril formation of amyloid beta 1-42 and/or removal from the extracellular environment and endoproteolysis of amyloid beta 1-42.

However, the relationship between the FPRL1 receptor and amyloid beta production/secretion has never been studied before, and the finding that FPRL1 increases amyloid beta 1-42 production/secretion in the conditioned medium of infected cells is completely novel.

EXAMPLE 2: Identification of close relatives of FPRL1 and GCGR.

The amino acid sequence of the human GCGR receptor was used as query in a BLAST search against all the human GPCRs in order to find its closest homologues. Table 5 shows the 5 closest homologues of the glucagon receptor. Using ClustalW, an alignment was constructed showing the degree of homology between GCGR and its closest homologues, the GLP1R and GLP2R (Figure 7).

The amino acid sequence of the human FPRL1 receptor was used as query in a BLAST search against all human GPCRs in order to find its closest homologues. Table 6 shows the 5 closest homologues of the FPRL1 receptor. Using ClustalW an alignment was constructed showing the degree of homology between the GCGR and its closest homologues, the FPR1 and FPRL2 (Figuré 8).

EXAMPLE 3: Functional analysis of GPCR receptors in HEK293 cells by reporter gene analysis.

All GPCRs share a common architecture of 7 transmembrane domains, an extracellular N-terminus and an intracellular C-terminus. The major signal transduction cascades activated by GPCRs are initiated by the activation of heterotrimeric G-proteins (Wess, 1998). In addition, minor signal transduction pathways that are G-protein independent exist (Marinissen and Gutkind, 2001). Heterotrimeric G-proteins are built from three different proteins: the G_α, G_β and G_γ subunits.

The signal transduction cascade starts with the activation of the receptor by an agonist. Transformational changes in the receptor are then translated down to the G-protein. The G-protein dissociates into the G_α subunit and the $G_{\beta\gamma}$ subunit. Both subunits dissociate from the receptor and are both capable of initiating different cellular responses. Best known are the cellular effects that are initiated by the Gα subunit.

It is for this reason that G-proteins are categorized by their Gα subunit. The G-proteins are divided into four groups: Gs, Gi/o, Gq and G12/13. Each of these G-proteins is capable of activating an effector protein, which results in changes in second messenger levels in the cell. The changes in second messenger level are the triggers that make the cell respond to the extracellular signal in a specific manner. Cellular responses range over a plethora of possibilities, from changes in cell shape to the transcriptional activation of genes.

The two most important second messengers in the cell are cAMP and Ca2+. The a-subunit of the Gs class of G-proteins is able to activate adenylyl cyclase, resulting in an increased turnover from ATP to cAMP. The a-subunit of Gi/o G-proteins does exactly the opposite and inhibits adenylyl cyclase activity resulting in a decrease of cellular cAMP levels. Together, these two classes of G-proteins regulate the second messenger cAMP. Ca2+ is regulated by the a-subunit of the Gq class of G-proteins. Through the activation of phospholipase C phosphatidylinositol 4,5-bisphosphate (PIP2) from the cell membrane are hydrolyzed to inositol 1,4,5-trisphosphate and 1,2-diacylglycerol, both these molecules act as second messengers. Inositol 1,4,5-trisphosphate binds specific receptors in the endoplasmatic reticulum, resulting in the opening of Ca2+ channels and release of Ca2+ in the cytoplasm.

Receptor activation can be measured by several different techniques. Usually these measurements detect the levels of second messengers either directly by ELISA or radioactive technologies or indirectly by reporter gene analysis. Reporter gene technology consists of an easily detectable gene, such as luciferase or b-galactosidase under the regulation of a promoter that responds to the cellular level of second messengers.

For the measurement of changes in cAMP levels we used a luciferase gene placed under the control of a minimal promoter regulated by cAMP responsive elements (CRE). In the cell, cAMP binds to the regulatory subunit of protein kinase A (PKA) and by forcing the subunit to dissociate from the catalytic subunit, cAMP activates PKA. cAMP responsive element binding protein (CREB) is one of the many substrates of PKA and is therefore

phosphorylated by PKA. Upon phosphorylation, CREB translocates to the nucleus and binds to CRE DNA sequences in promoter regions, initiating transcription of downstream genes. Activation of Gs by a GPCR will thus result in an increase in luciferase activity when the reporter gene construct is present in the same cell as the receptor. However, other signal transduction routes might also lead to activation of the CRE-reporter.

A similar reporter gene was constructed for the measurement of changes in intracellular Ca2+ levels. This reporter makes use of the Ca2+ dependent activation of the transcription factor NF-AT (nuclear factor activated T-cells). To activate this transcription factor, Ca2+ must activate calcineurin, which in turn acts as a phosphatase for NF-AT. The dephosphorylated form of NF-AT translocates to the nucleus and binds specific promoter elements. Binding of NF-AT to these cis-acting elements drives the transcription of a downstream gene, in our case the luciferase gene.

We have constructed both reporter gene constructs into an adenoviral vector. By doing so we can make an adenovirus and use this virus to introduce the reporter gene construct into our assay cells with the purpose to measure GPCR activation.

Adenoviruses were constructed harboring the luciferase gene under the control of a minimal promoter with CRE elements or NF-AT responsive elements, respectively. HEK293 cells were transduced with adenoviruses containing GPCRs and either the CRE reporter or the NF-AT reporter,.

In general, cells were plated in a 96 well plate at a density of 10,000 cells per well in Dulbeco´s

modified Eagles medium (DMEM) supplemented with 10% fetal calf serum (FCS). After the cells were firmly attached, GPCR or control viruses expressing eGFP and LacZ, were added to the cells at a MOI of 50. Subsequently reporter virus was added at an MOI of 400. The cells were incubated for 18 h with the virus before the virus was washed away and the medium replaced with DMEM, 5% FCS. The cells were left for an additional 24 h before they were treated with increasing amounts of agonist (glucagon or fMLF) for a period of 6 h after which the cells were lysed and the luciferase activity was measured using a luciferase detection kit such as the steady light kit from Packard according to the manufacturer's protocol.

Stimulation of GCGR with increasing amounts of glucagon showed a dose dependent increase in luciferase activity for both reporters, indicating that activation of the glucagon receptor results in an increase in CRE-reporter activity (Figure 5A) and NF-AT-reporter activity (Figure 5B). This result indicates that the glucagon receptor couples in HEK293 cells to Gq, giving rise to increased intracellular Ca2+- levels, while the stimulation of the CRE-reporter suggests cAMP-involvement or involvement of other signal transduction routes.

Stimulation of FPRL1 with increasing amounts of fMLFshowed a dose dependent decrease in luciferase activity indicating that activation of the FPRL1 receptor results in an decrease of intracellular cAMP (Figure 5C). Forskoline (10 mM) was added simultaneously with the ligand to increase the basal cAMP content of the cells so that a larger window of detection was

created. This result indicates that the formyl peptide receptor like 1 couples in HEK293 cells to Gi1-3 or Go.

EXAMPLE 4: Effect on amyloid beta peptide production by an agonist-activated G protein coupled receptor.

Whereas overexpression of GPCRs results in constitutive signaling, the activity of endogenous GPCRs is normally modulated by binding of natural occurring agonists or antagonists. Because this is why they are good drug targets, it is of great value for future therapeutic applications to show that amyloid beta levels can be modulated by the agonists or antagonists of the GPCRs.

Therefore, the effect of the fMLF (agonist for FPRL1) and glucagon peptides (agonist for GCGR) on amyloid beta levels were evaluated in the Hek293 APPwt cells. Hek293 APPwt cells were transduced with Ad5/empty, Ad5/GCGR and Ad5/FPRL1, respectively, at an MOI of 50 during 24 h. Viruses were washed away and fresh medium containing respectively 5nM glucagon and 1mM fMLF was added to the cells. 24h later, the conditioned medium was assayed in the amyloid beta 1-42 ELISA as described in Example 1. It was observed that the addition of 5nM glucagon to cells transduced with Ad5/GCGR resulted in a 2 fold increase of amyloid beta 1-42 levels compared to un-stimulated cells transduced with either Ad5/GCGR or Ad5/empty, indicating that an agonist of GCGR was able to modulate amyloid beta 1-42 levels (Figure 6A).

Similarly, stimulating Hek293 APPwt cells, that were transduced with Ad5/FPRL1, with 1mM fMLF yielded an increase in the amyloid beta 1-42 levels compared to un-

stimulated cells transduced with either Ad5/FPRL1 or Ad5/empty, indicating that an agonist of FPRL1 was able to modulate amyloid beta 1-42 levels (Figure 6B).

EXAMPLE 5: Expression of GPCRs in the human brain

Upon identification of a modulator of APP processing, it is of the highest importance to evaluate whether the modulator is expressed in the tissue and the cells of interest. This can be achieved by measuring the RNA and/or protein levels.

In recent years, RNA levels are being quantified through real time PCR technologies, whereby the RNA is first transcribed to cDNA and then the amplification of the cDNA of interest is monitored during a PCR reaction. The amplification plot and the resulting Ct value are indicators for the amount of RNA present in the sample. Determination of the levels of household keeping genes allows the normalization of RNA levels of the target gene between different RNA samples, represented as delta Ct values.

To assess whether the GPCRs of the invention are expressed in the human brain, real time PCR with GAPDH specific primers and specific primers for the GPCRs (Table 3) was performed on a dilution series of human total brain, human cerebral cortex, and human hippocampal total RNA (BD Biosciences). GAPDH was detected with a Taqman probe, while for the other GPCRs SybrGreen was used. In short, 40 ng of RNA was transcribed into DNA using the MultiScribe Reverse Transcriptase enzyme (Applied BioSystems) at 50 U/µl. The resulting cDNA was amplified with AmpliTaq Gold DNA

polymerase (Applied BioSystems) during 40 cycles using an ABI PRISM® 7000 Sequence Detection System.

Total brain, cerebral cortex and hippocampal total RNA were analyzed for the presence of GPCR transcripts of Table 1 via quantitative real time PCR.

For FPRL1, the obtained Ct values indicate that it was detected in all RNA samples (Table 4).

To gain more insight into the specific cellular expression, immunohistochemistry (protein level) was carried out on sections from a human normal brain hippocampal, cortical and subcortical structures (LifeSPan Biosciences, UK). These results indicated that FPRL1 expression occurs in neurons. The same approach can be followed for GCGR, to assess neuronal expression in human brain tissues. The comparison of diseased tissue with healthy tissue will teach us whether the GPCRs of the invention are expressed in the diseased tissue and whether their expression level is changed compared to the non-pathological situation.

EXAMPLE 6 Amyloid beta production in rat primary neuronal cells

In order to investigate whether GPCRs of the invention affect amyloid beta production in a real neuron, human or rat primary hippocampal or cortical neurons are treated with GPCR specific agonists and antagonists to activate or inhibit the endogenous GPCR. Alternatively, the expression levels of the GPCR can be increased via transduction of the cells with an adenovirus carrying the GPCR cDNA.

Since rodent APP genes carry a number of mutations in APP compared to the human sequence, they

produce less amyloid beta 1-40 and 1-42. In order, to achieve detectable amyloid beta levels, transduction with human wild type APP or human Swedish mutant APP (which enhances Abeta production) cDNA is necessary. Levels of secreted amyloid beta are determined by ELISA and mass spectrometry analyses (see Example 7).

Human primary neurons are obtained from Cellial Technologies, France. Rat primary neuron cultures are prepared from brain of E18-E19-day-old fetal Sprague Dawley rats according to Goslin and Banker (Culturing Nerve cells, second edition, 1998 ISBN 0-262-02438-1). Briefly, single cell suspensions obtained from the hippocampus or cortices are prepared. The number of cells is determined (only taking into account the living cells) and cells are plated on poly-L-lysine-coated plastic 96-well plates in minimal essential medium (MEM) supplemented with 10% horse serum. The cells are seeded at a density between 30,000 and 60,000 cells per well (i.e. about 100,000 - 200,000 cells/cm2, respectively). After 3-4 h, culture medium was replaced by 150 µl serum-free neurobasal medium with B27 supplement (GIBCO BRL). Cytosine arabinoside (5 µM) was added 24 h after plating to prevent nonneuronal (glial) cell proliferation.

Neurons are infected at day 3 after plating. Before adenoviral transduction, 150 µl of conditioned medium of these cultures is transferred to the corresponding wells in an empty 96-well plate and 50 µl of the conditioned medium returns to the cells. The remaining 100 µl/well is stored at 37°C and 5% CO2. Both hippocampal and cortical primary neuron cultures are infected with the crude lysate of virus containing the cDNAs of the human wild type APP or human Swedish mutant

APP at MOI 2000. Sixteen to twenty-four hours after transduction, virus is removed and cultures are washed with 100 µl pre-warmed fresh neurobasal medium. After removal of the wash solution, the remaining 100 µl of the stored conditioned medium is transferred to the corresponding cells. The cells are treated at either day 4, day 5, day 6 or day 7 with the agonists and antagonists at different concentrations.

From now on, cells accumulate amyloid beta in the conditioned medium and its concentration is determined by amyloid beta 1-42 and amyloid beta x-42 specific ELISA's (see Example 1). The conditioned media are collected 6, 12, 24, 48 and 96 hours after start of the treatment.

Stimulating the GPCR with the agonist results in an increase in amyloid beta peptides, which is inhibited by addition of the antagonist to the cells. The use of the GPCR specific agonists and antagonists, confirms its involvement in APP processing.

EXAMPLE 7 Amyloid beta peptides profiling in conditioned medium of HEK293 APP770wt cells and rat primary neuronal cells using Mass Spectrometry

To specify how APP processing is exactly modulated by GPCRs of the present invention, a mass spectrometry analysis is carried out on the conditioned medium of cells overexpressing the GPCRs, or cells into which the activity of the endogenous GPCR is inhibited with its antagonist, to identify the inhibited amyloid beta peptide species.

T25 flasks (Cellstar, Greiner Bio-One) are coated with collagen (5 µg/ml) for 4h at 37°C. After

replacement of the collagen by medium (DMEM from GIBCO with 10% FBS from ICN), HEK293 APP770wt cells are seeded at a density of 3.106 cells per flask. Cells are grown overnight at 37°C, 10% CO2. Next day, cells are infected with the crude lysate of virus containing the cDNAs of the GPCRs at the appropriate MOI. The cells are incubated at 37°C, 10% CO2. After 12 to 24 hours, the cell culture medium is removed by aspiration and 3 ml of fresh medium (DMEM, 0.2% FBS, 1X ITS from GIBCO) is added to the cells. 24 hours later, the conditioned medium is harvested. Protease inhibitors are added immediately and the samples are kept on ice in Falcon tubes until further processing.

Of each sample, 850 µl of the conditioned medium is transferred to an Eppendorf tube in triplet. After rigorously vortexing the Protein G Sepharose beads, 5 µl of the slurry is added to each tube, together with 1 µg of specific antibody e.g. 4G8 or JRF/cAbeta42/26 (obtained from M Mercken, Johnson and Johnson Pharmaceutical Research and Development, B-2340 Beerse, Belgium). Tubes are rotated overnight at 4°C and centrifuged for 10 min. All centrifuge steps are at 13200 rpm at 4°C. After aspiration of the supernatant, beads are washed twice by adding 850 µl of wash buffer (10 mM Tris-HCl (pH 8.0) containing 0.1% n-octylglucoside, 150 mM NaCl, 0.025% sodium azide) and centrifuging for 10 min. After a final wash step with 850 µl of 10 mM Tris-HCl (pH 8.0), cells are centrifuged for 10 min and supernatant is removed completely. Dry pellets are stored at -80°C until further analysis.

A saturated solution of matrix (alpha-cyano-hydroxy-cinnamic acid, HCCA) is prepared in 500 µl acetonitrile by vortexing. After adding 400 µl water and

100 µl 1% trifluoroacetic acid, the tube is vortexed for 3 min. This results in 50% acetonitrile/0.1% TFA matrix containing elution buffer. 3.5 µl of this elution buffer is added to 5 µl of thawed dry beads and sonicated for 1 min in a water bath at room temperature. The samples are briefly spun (30 s) at maximal speed (14.000 rpm).

One µl of eluted sample is directly spotted on a ground stainless steel MALDI target plate. Samples are allowed to air dry until crystallization of sample. The target plate is inserted into the MALDI-TOF-TOF mass spectrometer and measurements are performed according to the MALDI-TOF instructions. The resulting spectra are calibrated using a standard curve acquired using a mixture of several standard peptides obtained from Sigma. These standard peptides are in the mass range of 1200 - 3200 Da.

EXAMPLE 8: Ligand screen for GPCRs

Reporter gene screen.

Mammalian cells such as HEK293 or CHO-K1 cells are either stably transfected with a plasmid harboring the luciferase gene under the control of a cAMP dependent promoter (CRE elements) or transduced with an adenovirus harboring a luciferase gene under the control of a cAMP dependent promoter.

In addition reporter constructs can be used with the luciferase gene under the control of a Ca2+ dependent promoter (NF-AT elements) or a promoter that is controlled by activated NF-kB. These cells, expressing the reporter construct, are then transduced with an adenovirus harboring the cDNA of the GPCR of the present invention. 40 h after transduction the cells are

treated with an agonist for the receptor (Tables 7 and 8) and screened against a large collection of reference compounds comprising peptides (LOPAP, Sigma Aldrich), lipids (Biomol, TimTech), carbohydrates (Specs), natural compounds (Specs, TimTech), and small chemical compounds (Tocris).

Compounds, which decrease the agonist induced increase in luciferase activity, are considered to be antagonists or inverse agonists for the GPCR they are screened for. These compounds are screened again for verification and screened against their effect on secreted amyloid beta peptide levels.

In addition, cells expressing the NF-AT reporter gene can be transduced with an adenovirus harboring the cDNA encoding the a-subunit of G15 or chimerical G_α subunits. G15 is a promiscuous G protein of the G_q class that couples to many different GPCRs and as such re-directs their signaling towards the release of intracellular Ca^{2+} stores. The chimerical G alpha subunits are members of the G_s and $G_{i/o}$ family by which the last 5 C-terminal residues are replaced by those of $G_{\alpha q}$, these chimerical G-proteins also redirect cAMP signaling to Ca^{2+} signaling.

FLIPR screen.

Mammalian cells such as HEK293 or CHO-K1 cells are stably transfected with a expression plasmid construct harboring the cDNA of a GPCR of the present invention. Cells are seeded and grown until sufficient stable cells can be obtained. Cells are loaded with a Ca2+ dependent fluorophore such as Fura3 or Fura4. After washing away the excess of fluorophore the cells are

screened against a large collection of reference compounds comprising peptides (LOPAP, Sigma Aldrich), lipids (Biomol, TimTech), carbohydrates (Specs), natural compounds (Specs, TimTech), and small chemical compounds (Tocris) by simultaneously adding an agonist (Table 7) and a compound to the cells. As a reference just the agonist is added. Activation of the receptor is measured as an almost instantaneously increase in fluorescence due to the interaction of the fluorophore and the Ca2+ that is released. Compounds that reduce or inhibit the agonist induced increase in fluorescence are considered to be antagonists or inverse agonists for the receptor they are screened against. These compounds will be screened again to measure the secreted amyloid beta peptide.

AequoScreen.

CHO cells, stably expressing Apoaequorin are stably transfected with a plasmid construct harboring the cDNA of a GPCR. Cells are seeded and grown until sufficient stable cells can be obtained. The cells are loaded with coelenterazine, a cofactor for apoaequorin. Upon receptor activation intracellular Ca2+ stores will be emptied and the aequorin will react with the coelenterazine in a light emitting process.

The emitted light is a measure for receptor activation. The CHO, stable expressing both the apoaequorin and the receptor are screened against a large collection of reference compounds comprising peptides (LOPAP, Sigma Aldrich), lipids (Biomol, TimTech), carbohydrates (Specs), natural compounds (Specs, TimTech), and small chemical compounds (Tocris)

by simultaneously adding an agonist and a compound to the cells. As a reference just the agonist is added. Activation of the receptor is measured as an almost instantaneously light flash due to the interaction of the apoaequorin, coelenterazine and the Ca2+ that is released. Compounds that reduce or inhibit the agonist induced increase in light are considered to be antagonists or inverse agonists for the receptor they are screened against. These compounds will be screened again for verification and secreted amyloid beta levels.

In addition, CHO cells stable expressing the apoaequorin gene are stably transfected with a plasmid construct harboring the cDNA encoding the a-subunit of G15 or chimerical G_{α} subunits. G15 is a promiscuous G protein of the G_q class that couples to many different GPCRs and as such redirect their signaling towards the release of intracellular Ca^{2+} stores. The chimerical G alpha subunits are members of the G_s and $G_{i/o}$ family by which the last 5 C-terminal residues are replaced by those of $G_{\alpha q}$, these chimerical G-proteins also redirect cAMP signaling to Ca^{2+} signaling.

Screening for compounds that bind to the polypeptides of the present invention

Compounds are screened for binding to the polypeptides of the present invention. The affinity of the compounds to the polypeptides is determined in a displacement experiment. In brief, the polypeptides of the present invention are incubated with a labeled (radiolabeled, fluorescent labeled) ligand that is known to bind to the polypeptide and with an unlabeled compound. The displacement of the labeled ligand from

the polypeptide is determined by measuring the amount of labeled ligand that is still associated with the polypeptide. The amount associated with the polypeptide is plotted against the concentration of the compound to calculate IC50 values. This value reflects the binding affinity of the compound to its target, i.e. the polypeptides of the present invention. Strong binders have an IC50 in the nanomolar and even picomolar range. Compounds that have an IC50 of at least 10 micromol or better (nmol to pmol) are applied in beta amyloid secretion assay to check for their effect on the beta amyloid secretion and processing. The polypeptides of the present invention can be prepared in a number of ways depending on whether the assay will be run on cells, cell fractions or biochemically, on purified proteins.

Receptor ligand binding study on cell surface The receptor is expressed in mammalian cells (HEK293, CHO, COS7) cells by adenovirally transducing the cells (see US 6,340,595). The cells are incubated with both labeled ligand (iodinated, tritiated, or fluorescent) and the unlabeled compound at various concentrations, ranging from 10 pM to 10mM (3 hours at 4°C.: 25 mM HEPES, 140 mM NaCl, 1 mM CaCl2, 5 mM MgCl2 and 0.2% BSA, adjusted to pH 7.4). Reactions mixtures are aspirated onto PEI-treated GF/B glass filters using a cell harvester (Packard). The filters are washed twice with ice cold wash buffer (25 mM HEPES, 500 mM NaCl, 1 mM CaCl2, 5 mM MgCl2, adjusted to pH 7.4). Scintillant (MicroScint-10; 35 µl) is added to dried filters and the filters counted in a (Packard Topcount) scintillation counter. Data are analyzed and plotted using Prism software (GraphPad Software, San Diego, Calif.).

Competition curves are analyzed and IC50 values calculated. If 1 or more data points do not fall within the sigmoidal range of the competition curve or close to the sigmoidal range the assay is repeated and concentrations of labeled ligand and unlabeled compound adapted to have more data points close to or in the sigmoidal range of the curve.

Receptor ligand binding studies on membrane preparations

Membranes preparations are isolated from mammalian cells (HEK293, CHO, COS7) cells overexpressing the receptor is done as follows: Medium is aspirated from the transduced cells and cells are harvested in 1 x PBS by gentle scraping. Cells are pelleted (2500 rpm 5 min) and resuspended in 50 mM Tris pH 7.4 (10 x 10E6 cells/ml). The cell pellet is homogenized by sonicating 3 x 5 sec (UP50H; sonotrode MS1; max amplitude: 140 µm; max Sonic Power Density: 125W/cm2). Membrane fractions are prepared by centrifuging 20 min at maximal speed (13000 rpm ~15 000 to 20 000g or rcf). The resulting pellet is resuspended in 500 µl 50 mM Tris pH 7.4 and sonicated again for 3 x 5 sec. The membrane fraction is isolated by centrifugation and finally resuspended in PBS. Binding competition and derivation of IC50 values are determined as described above.

EXAMPLE 9: Inhibition of the GPCR mediated effect on amyloid beta production via knock down of the GPCR expression levels.

The effect of an antagonist can be mimicked through the use of siRNA based strategies, which will

result in decreased expression levels of the targeted protein. Adenoviral mediated siRNA or knock down constructs based upon the sequences shown in Table 9, were constructed as described in WO03020931.

SH-SY5Y Cells were seeded in collagen-coated plates in 50 ml, at a cell density of 15000 cells/well (384 well plate) in DMEM 10%FBS containing 1mM 9 cis-retinoic acid. 48 h later, 10 ml of fresh DMEM 10%FBS containing 1mM 9 cis-retinoic acid was added and the cells were infected at a multiplicity of infection (MOI) of 1250; 625; 250; 50 and 10 with adenovirus containing knock down sequences targeted against eGFP or FPRL1 (FPRL1_v6 = _Seq ID # 53; FPRL1_v7 = Seq ID # 46. In addition, an adenovirus harboring the APPsw cDNA was infected at an MOI of 500. The following day, the viruses were washed away with 80 ml DMEM 10%FBS containing 1mM 9 cis-retinoic acid and 80 ml DMEM 10%FBS containing 1mM 9 cis-retinoic acid was added to the cells. After 96 h, the medium was refreshed with 80 ml DMEM 10%FBS containing 1mM 9 cis-retinoic acid and 0.025 mM Hepes. Amyloid beta peptides were allowed to accumulate during 48h. The levels of the amyloid beta 1-42 peptides were determined with the amyloid beta 1-42 ELISA as described in example 1. This experiment clearly shows that knock-down constructs targeted against FPRL1 reduce amyloid beta 1-42 levels in the conditioned medium of SH-SY5Y cells, underscoring its involvement in amyloid beta 1-42 production (see Figure 18).

EXAMPLE 10: Functional analysis of FPRL1_v2 in HEK293 cells by reporter gene analysis.

As described in Example 3, cells were plated in a 96 well plate at a density of 10,000 cells per well in Dulbeco´s modified Eagles medium (DMEM) supplemented with 10% fetal calf serum (FCS). After the cells were firmly attached, FPRL1_v2 or empty control viruses were added to the cells with a MOI of 50. Subsequently reporter virus was added at an MOI of 400. The cells were incubated for 18 h with the virus before the virus was washed away and the medium replaced with DMEM, 5% FCS. The cells were left for an additional 24 h before they were treated with increasing amounts of WKYMVm peptide for a period of 6 h after which the cells were lysed and the luciferase activity was measured using the steady light kit from Packard or luciferase kits from other suppliers according to the manufacturer's protocol.

Stimulation of FPRL1_v2 with increasing amounts of WKYMVm showed a dose dependent decrease in luciferase activity indicating that activation of the FPRL1 receptor results in an decrease of the CRE reporter (Figure 15). NKH477 (water soluble analogue of forskolin) (10 mM) was added simultaneously with the ligand to increase the basal cAMP content of the cells so that a larger window of detection was created. This result indicates that the FPRL1_v2 couples in HEK293 cells to Gi/o G-proteins. At high concentrations of WKYMVm an increase in luciferase activity is observed. Studies using the NFAT reporter (Ca2+) indicated that at over 100nM concentrations of WKYMVm the FPRL1_v2 receptors couples to the Gq class of G-proteins.

In addition, Abeta 1-42 was determined in the conditioned medium of HEK293 APP cells infected with FPRL1_v2 after being stimulated with WKYMVm and 10 µM

NKH477. As described in Example 3, cells were plated in a 96 well plate at a density of 10,000 cells per well in Dulbeco´s modified Eagles medium (DMEM) supplemented with 10% fetal calf serum (FCS). After the cells were firmly attached, FPRL1_v2 or empty control viruses were added to the cells with a MOI of 50. The cells were incubated for 18 h with the virus before the virus was washed away and the medium replaced with DMEM, 5% FCS. The cells were left for an additional 24 h before they were treated with increasing amounts of WKYMVm peptide for a period of 24 h before the conditioned medium was collected. The beta-amyloid concentration in the medium was determined as described in Example 1. As shown in Figure 16, the WKYMVm peptide increases dose dependently the amyloid beta secretion in HEK293 cells transduced with the FPRL1_v2 receptor.

EXAMPLE 11: Validation of FPRL1_v2 in HEK293 cells by assessing APP processing.

APP processing is mediated by the subsequent activity of beta-secretase and gamma-secretase. As illustrated in Figure 1, APP cleavage by beta-secretase yields the production of an N-terminal sAPPbeta and a C-terminal beta-CTF. Both APP fragments can be determined by appropriate assays that are easy to apply for those skilled in the art. Cell lysates of HEK293 APPwt cells prior infected with either FPRL1_v1, FPRL1_v2 or LacZ were loaded on a PAGE gel and the beta-CTFs were visualized by incubating the corresponding blot with the highly specific, high affinity WO-2 antibody (The Genetics Company). Both FPRL1 versions generated a dose dependent increase in beta-CTF compared to the LacZ

control (Figure 11). The conditioned media of these cultures were used to determine the levels of secreted APPbeta (sAPPbeta). For this purpose, a polyclonal antibody was generated in house specifically recognizing secreted APPbeta derived from wild-type APP. This antibody was used in a sandwich ELISA (coating antibody was the polyclonal sAPPbeta antibody; detection antibody was the monoclonal APP13M (Alpha Diagnostics)) to reveal that both FPRL1 versions increased the levels in sAPPbeta dose-dependently (Figure 12). These data let us assume that FPRL1 is a beta-secretase modulator.

Once sAPPbeta and beta-CTF have been formed, gamma-secretase cleaves beta-CTF to form Abeta and a shorter C-terminal fragment. To assess whether FPRL1 is able to modulate gamma-secretase activity, HEK293 cells were infected with Ad5/C99. Using this virus, one over-expresses the beta-CTF (also called C99) in the HEK293 cells. These cells were simultaneously infected with Ad5/FPRL1. As illustrated in Figure 13, both FPRL1 versions triggered the production of Abeta 1-42, meaning that FPRL1 was able to increase the gamma-secretase activity as well. In addition, the cell lysates of Ad5/FPRL1 infected HEK293 APP cells were used to assess the levels of full length APP. Overexpression of both FPRL1_v1 and FPRL1_v2 increased the levels of mature full length APP and this level of increase was related to the level of expression of FPRL1 (Figure 14).

EXAMPLE 12: Validation of FPRL1 in HEK293 cells by assessing Abeta production when cells are challenged with FPRL1 specific agonists and antagonists.

Antagonists for FPRL1 were tested to evaluate whether inhibiting the FPRL1 receptor results in a decrease of the amyloid beta 1-42 levels. Hek293 APPwt cells were infected with respectively Ad5/empty, and Ad5/FPRL1_v2 over 24 h. Viruses were washed away and fresh medium containing increasing amounts of WKYMVm ((Seq ID no 357) in the absence and presence of fixed (2 and 20 µM) concentrations of WRWWWW antagonist ((Seq ID no 358)) was added to the cells. 24h later, the conditioned medium was assayed in the amyloid beta 1-42 ELISA as described in Example 1. As shown in Figure 17, the WRWWWW antagonist reduced the amount of Abeta 1-42 secreted in the medium caused by the overexpression of FPRL1_v2 and incubation with WKYMVm in HEK293 Appwt cells.

CLAIMS

1. Use of an antagonist of a polypeptide selected from the group consisting of SEQ ID Nos.: 15-28 for the preparation of a medicament for prevention and/or treatment, by reducing the level of amyloid-beta protein, of diseases involving cognitive impairment.

2. Use according to claim 1, wherein the polypeptide is SEQ ID No: 15.

3. Use according to claim 1 or claim 2, wherein the disease is Alzheimer's disease.

4. Use according to any of the claims 1-3, wherein the antagonist is selected from the group consisting of a peptide comprising the amino acid sequence WRWWWW; chenodeoxycholic acid; cyclosporin (Cs) H; BocPLPLP; Glucagon derivatives; [desHis(1)-[Glu(9)]-glucagon-amide;
[desHis(1), Ala(4), Glu(9)] glucagon amide; [desHis(1), D-Ala(4), Glu(9)] glucagon amide; [desHis(1), Leu(4), Glu(9)] glucagon amide; [desHis(1), D-Leu(4), Glu(9)] glucagon amide; NNC 92-1687; BAY 27-9955; alkylidene hydrazide derivatives with alkoxyaryl moieties; [4-hydroxy-3-cyanobenzoic acid (4-isopropylbenzyloxy-3,5-dimethoxymethylene)hydrazide]; 3-cyano-4-hydroxybenzoic acid [1-(2,3,5,6-tetramethylbenzyl)-1H-indol-4-ylmethylene]hydrazide; non-peptide glucagons receptor antagonists; quinoxalines /pyrrolo[1,2 -a]quinoxalines; mercaptobenzimidazoles; 2-pyridyl-3,5-diarylpyrroles; quinoline hydrazones; 4-phenylpyridines; 5-hydroxyalkyl-4-phenylpyridines; triarylimidazole and triarylpyrrole

antagonists; an antibody or a fragment thereof; and 2-(-4-Pyridyl)-5-(4-chlorophenyl)-3-(5-bromo-2-propyloxyphenyl)pyrrole.

5. Use of a compound inhibiting the translation of a polynucleotide sequence selected from the group consisting of SEQ ID Nos.: 1-14 into a polypeptide according to SEQ ID Nos: 15-28, receptively, for the preparation of a medicament for prevention and/or treatment, by reducing the level of amyloid-beta protein, of diseases involving cognitive impairment.

6. Use according to claim 5, wherein the nucleotide sequence is SEQ ID No: 1.

7. Use according to claim 5 or claim 6, wherein the disease is Alzheimer's disease.

8. Use according to any of the claims 5-7, wherein the compound is selected from the group consisting of an antisense RNA, a ribozyme that cleaves the polynucleotide, an antisense oligodeoxynucleotide (ODN), a small interfering RNA (siRNA), and an antibody or fragment thereof reactive to the polynucleotide.

9. Use according to claim 8, wherein the siRNA comprises a sense strand of 17-23 nucleotides homologous to a nucleotide sequence selected from the group consisting of SEQ ID Nos: 1-14 and an antisense strand of 17-23 nucleotides complementary to the sense strand.

10. Use according to claim 9, wherein the siRNA further comprises a loop region connecting the sense and

the antisense strand.

11. Use according to claim 10, wherein the loop region consist of the nucleic acid sequence defined by SEQ ID No: 339.

12. Use according to any of the claims 8-11, wherein the siRNA comprises a sense nucleotide sequence selected from the group consisting of SEQ ID Nos: 29-338.

13. Use according to any of the claims 5-12, wherein the inhibiting compound is included within a vector.

14. Use according to claim 13, wherein the vector is an adenoviral, retroviral, adeno-associated viral, lenti viral or a sendai viral vector.

15. Method for identifying an antagonist of a polypeptide selected from the group consisting of SEQ ID Nos.: 15-28 or a compound inhibiting the translation of a polynucleotide sequence selected from the group consisting of SEQ ID Nos.: 1-14 into a polypeptide according to SEQ ID Nos: 15-28, receptively, comprising:

(a) providing a host cell expressing a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID Nos: 15-28, or a fragment, or a derivative thereof;

(b) determining a first activity level of the polypeptide by measuring the level of one or more second

messengers of the polypeptide;

(c) exposing the host cell to a compound;

(d) determining a second activity level of the polypeptide by measuring the level of the second messengers after exposing of the compound; and

(e) identifying an antagonist or an inhibiting compound by identifying the compound according to step (c) that provides a difference between the first and the second activity level.

16. Method according to claim 15 comprising contacting the host cell with an agonist for the polypeptide before determining the first activity level according to step (b).

17. Method according to claim 15 or 16 further comprising:

(f) contacting a population of mammalian cells expressing a polypeptide having a amino acid sequences selected from the group consisting of SEQ ID NO: 15-28, or a fragment, or a derivative thereof with the antagonist or the inhibiting compound identified in step (e)

(g) identifying the antagonist or inhibiting compound that reduces the amyloid-beta protein secretion by the cells.

18. Method according to any of the claims 15-17, wherein the polypeptide is SEQ ID No: 15 (FPRL1), encoded by SEQ ID NO: 1.

19. Method according to any of the claims 15-17, wherein the polypeptide is SEQ ID No: 22 (GCGR), encoded by SEQ ID NO: 8.

20. Method according to any of claims 15-19, wherein the activity level is determined with a reporter controlled by a promoter which is responsive to the second messenger.

21. Method according to claim 20, wherein the promoter is a cyclic AMP-responsive promoter, an NF-KB responsive promoter, or a NF-AT responsive promoter.

22. Method according to claim 20 or claim 21, wherein the reporter is luciferase or beta-galactosidase.

23. Method for identifying an antagonist of a polypeptide selected from the group consisting of SEQ ID Nos.: 15-28 or a compound inhibiting the translation of a polynucleotide sequence selected from the group consisting of SEQ ID Nos.: 1-14 into a polypeptide according to SEQ ID Nos: 15-28, receptively, comprising:

(a) contacting a compound with a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID Nos: 15-28, or a derivative, or a fragment thereof, or with a polynucleotide sequence or a

vector comprising a nucleic acid sequence selected from the group consisting of SEQ ID Nos: 1-14;

(b) determining the binding affinity of the compound to the polypeptide or the polynucleotide sequence;

(c) contacting a population of mammalian cells expressing the polypeptide according to SEQ ID. Nos. 15-28 with the compound that exhibits a binding affinity of 10 micromolar or less, and

(d) identifying an antagonist or an inhibiting compound by identifying the compound that provides a decrease in the level of amyloid-beta protein secretion by the mammalian cells.

24. Method according to any of the claims 15-23, wherein the antagonist or inhibiting compound is a low molecular weight antagonist or compound.

25. Method according to any of the claims 15-23, wherein the antagonist or inhibiting compound is a peptide.

26. Method according to any of the claim 15-23, wherein the antagonist or inhibiting compound is a lipid.

27. Method according to any of the claim 15-23, wherein the antagonist or the inhibiting compound is a natural compound.

28. Polynucleotide sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338.

29. Polynucleotide sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338 for use as a medicament.

30. Vector comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338.

31. Vector comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 29-338 for use as a medicament.

32. Method for diagnosing a pathological condition involving cognitive impairment or a susceptibility to the condition in a subject comprising:

(a) obtaining a sample of the subject's mRNA corresponding to a nucleic acid selected from the group consisting of SEQ ID Nos: 1-14 or a sample of the subject's genomic DNA corresponding to a genomic sequence of a nucleic acid selected from the group consisting of SEQ ID Nos: 1-14;

(b) determining the nucleic acid sequence of the subject's mRNA or genomic DNA;

(c) comparing the nucleic acid sequence of the subject's mRNA or genomic DNA with a nucleic acid selected from the group consisting of SEQ ID Nos: 1-14 or with a genomic sequence encoding a nucleic

acid selected from the group consisting of SEQ ID Nos: 1-14 obtained from a database; and

(d) identifying any difference(s) between the nucleic acid sequence of the subject's mRNA or genomic DNA and the nucleic acid selected from the group consisting of SEQ ID Nos: 1-14 or the genomic sequence encoding a nucleic acid selected from the group consisting of SEQ ID Nos: 1-14 obtained from a database.

33. Method for diagnosing a pathological condition involving cognitive impairment or a susceptibility to the condition in a subject, comprising determining the amount of polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID Nos: 15-28 in a biological sample, and comparing the amount with the amount of the polypeptide in a healthy subject, wherein an increase of the amount of polypeptide compared to the healthy subject is indicative of the presence of the pathological condition.

34. Method according to claim 32 or claim 33, wherein the pathological condition is Alzheimer's disease.

Figure 1

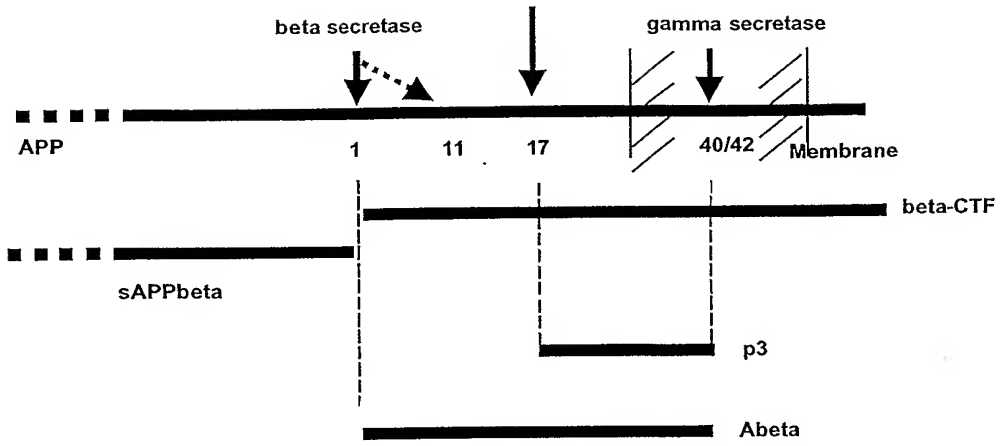
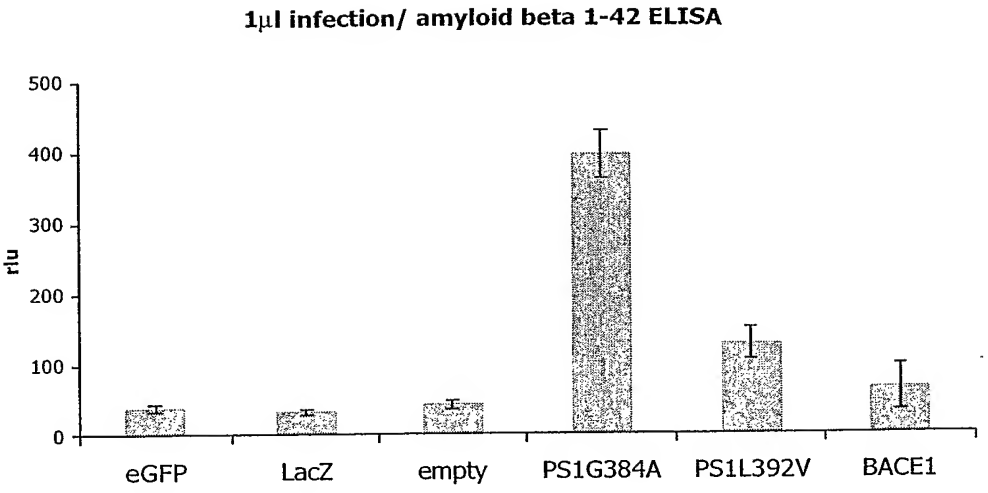

beta secretase
gamma secretase
APP
1
11
17
40/42
Membrane
beta-CTF
sAPPbeta
p3
Abeta

Figure 2

A


1µl infection/ amyloid beta 1-42 ELISA
500
400
300
200
100
0
rlu
eGFP
LacZ
empty
PS1G384A
PS1L392V
BACE1

B

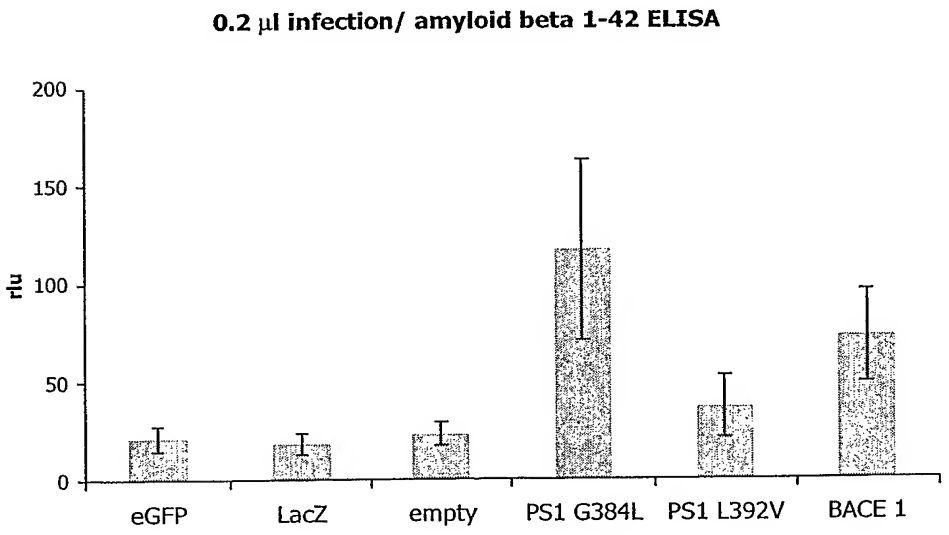

0.2 µl infection/ amyloid beta 1-42 ELISA
200
150
100
50
0
rlu
eGFP
LacZ
empty
PS1 G384L
PS1 L392V
BACE 1

Figure 3

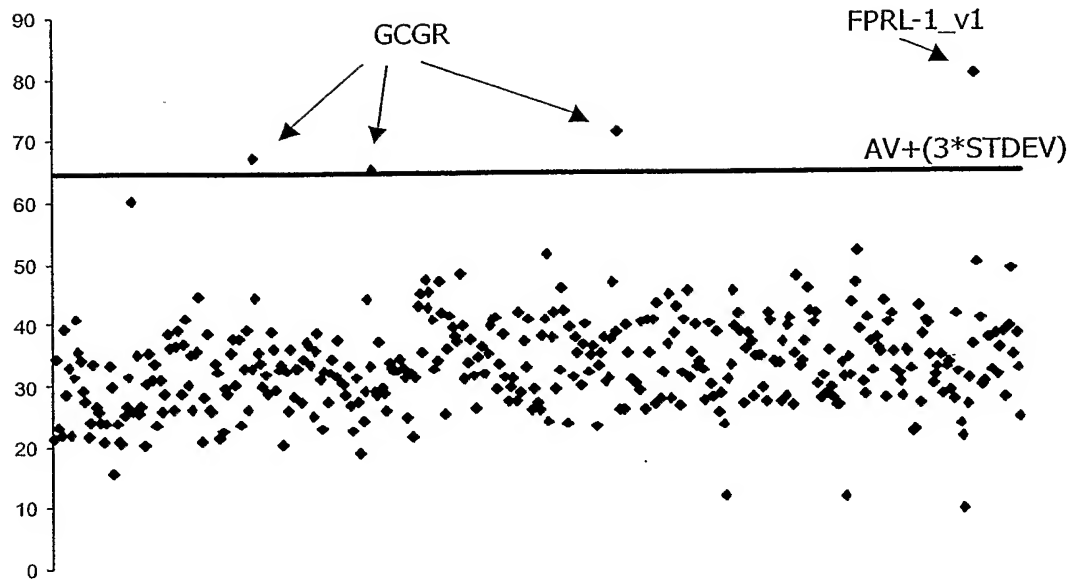
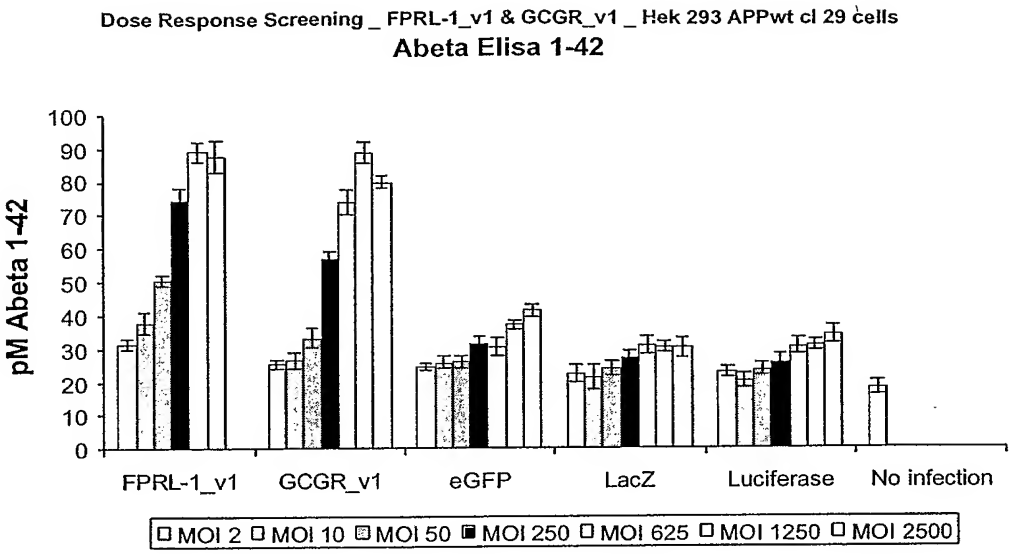


Figure 4

A


Dose Response Screening _ FPRL-1_v1 & GCGR_v1 _ Hek 293 APPwt cl 29 cells
Abeta Elisa 1-42
pM Abeta 1-42
100
90
80
70
60
50
40
30
20
10
0
FPRL-1_v1
GCGR_v1
eGFP
LacZ
Luciferase
No infection
MOI 2
MOI 10
MOI 50
MOI 250
MOI 625
MOI 1250
MOI 2500

B

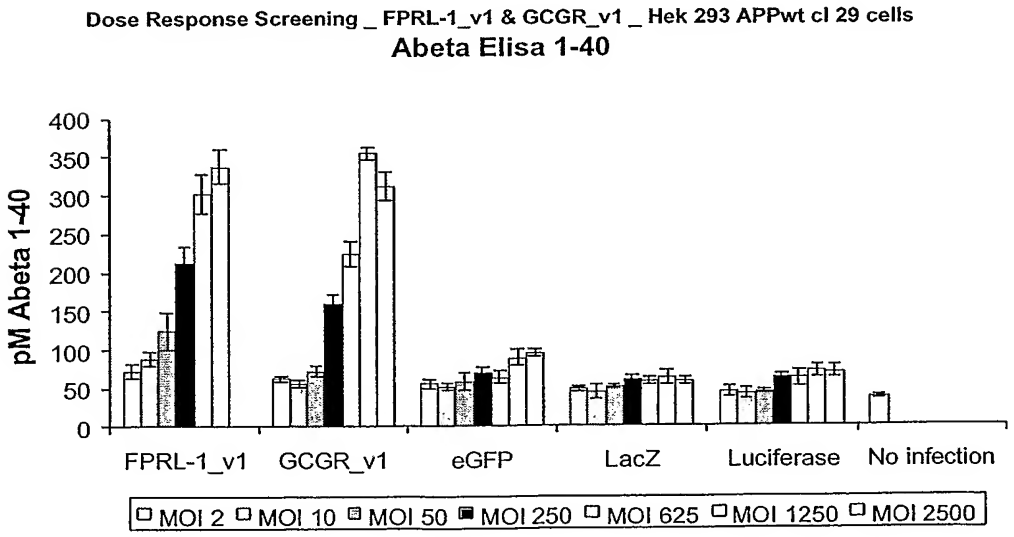

Dose Response Screening _ FPRL-1_v1 & GCGR_v1 _ Hek 293 APPwt cl 29 cells
Abeta Elisa 1-40
pM Abeta 1-40
400
350
300
250
200
150
100
50
0
FPRL-1_v1
GCGR_v1
eGFP
LacZ
Luciferase
No infection
MOI 2
MOI 10
MOI 50
MOI 250
MOI 625
MOI 1250
MOI 2500

Figure 4 (continued)

C

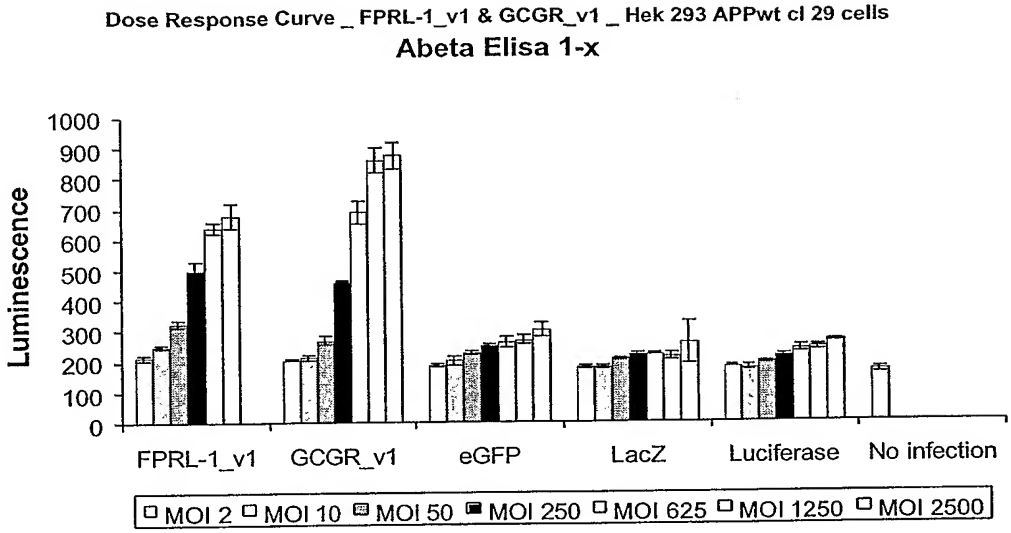
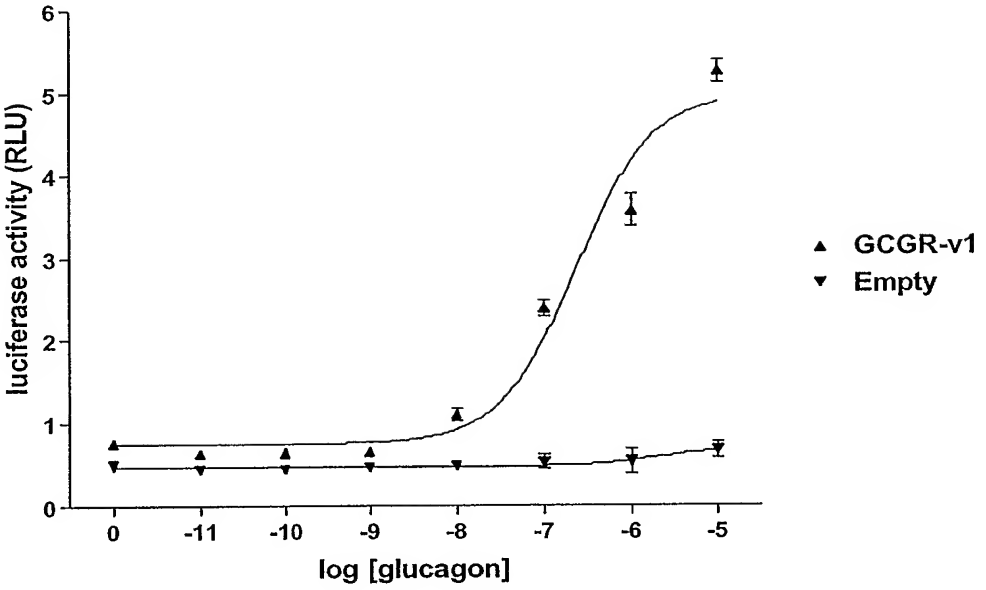


Figure 5

A


luciferase activity (RLU)
6
5
4
3
2
1
0
0
-11
-10
-9
-8
-7
-6
-5
log [glucagon]
GCGR-v1
Empty

B

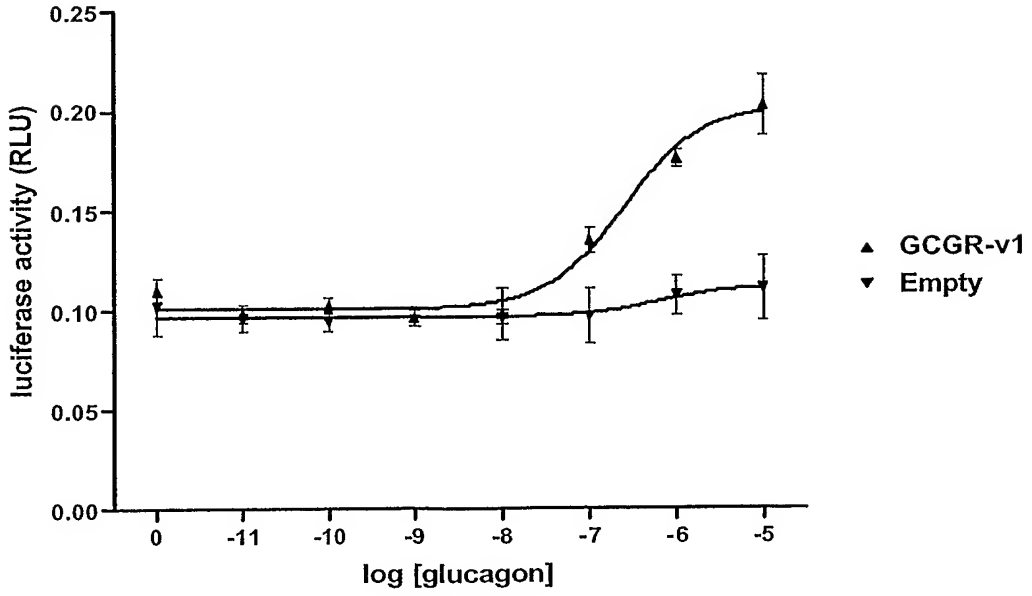

luciferase activity (RLU)
0.25
0.20
0.15
0.10
0.05
0.00
0
-11
-10
-9
-8
-7
-6
-5
log [glucagon]
GCGR-v1
Empty

Figure 5 (continued)

C

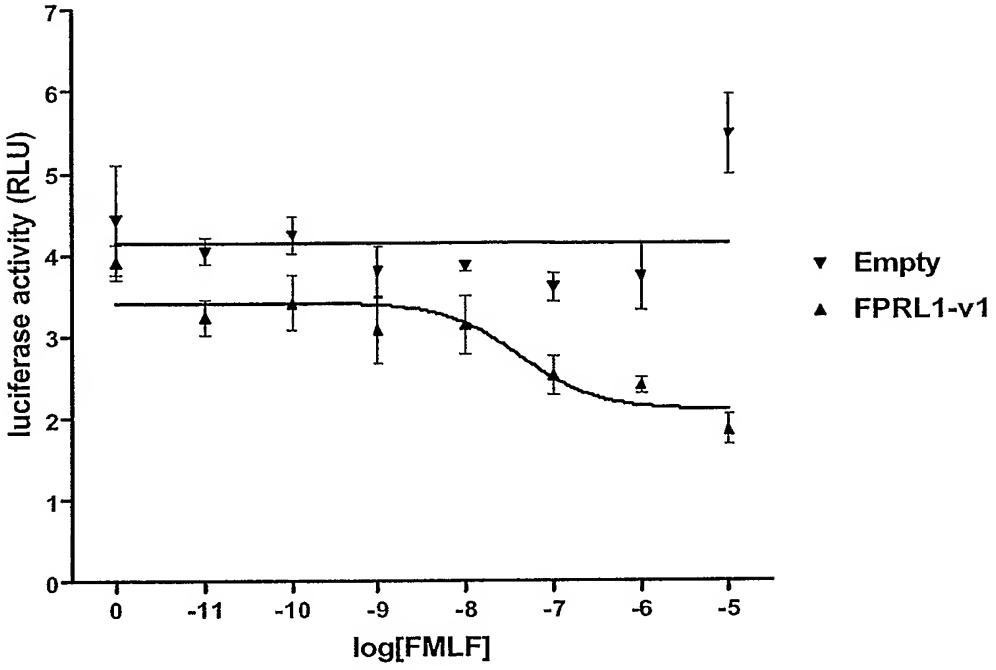


Figure 6

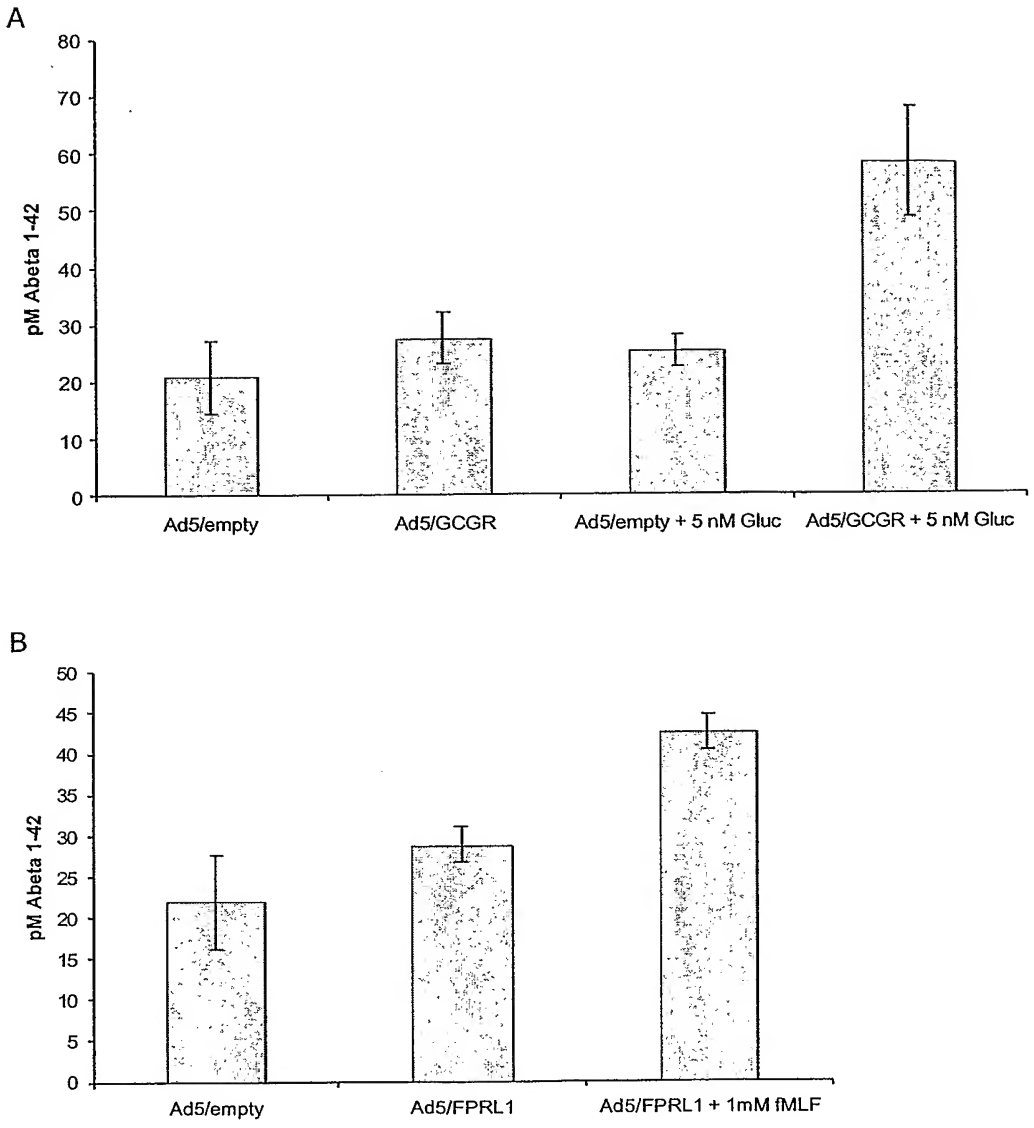

A
pM Abeta 1-42
80
70
60
50
40
30
20
10
0
Ad5/empty
Ad5/GCGR
Ad5/empty + 5 nM Gluc
Ad5/GCGR + 5 nM Gluc
B
pM Abeta 1-42
50
45
40
35
30
25
20
15
10
5
0
Ad5/empty
Ad5/FPRL1
Ad5/FPRL1 + 1mM fMLF

Figure 7

A.
Clustal W alignment of GCGR peptide sequence with GLP1R peptide sequence.

```
GLP1R  1    MAGAPGPLRL ALLLLGMVGR AGPRPQGATV SLWETVQKWR EYRRQCQRSL TEDPPPATDL  60
GCGR   1    MPPCQ-PQRP LLLLLLLLAC QPQVPSAQVM DF--LFEKWK LYGDQCHHNL SLLPPP-TEL  56

GLP1R  61   FCNRTFDEYA CWPDGEPGSF VNVSCPWYLP WASSVPQGHV YRFCTAEGLW LQKDNSSLPW  120
GCGR   57   VCNRTFDKYS CWPDTPANTT ANISCPWYLP WHHKVQHRFV FKRCGPDGQW VR-GPRGQPW  115

GLP1R  121  RDLSECEESK RGERSSPEEQ LLFLY----I IYTVGYALSF SALVIASAIL LGFRHLHCTR  176
GCGR   116  RDASQCQMD- -GEEIEVQKE VAKMYSSFQV MYTVGYSLSL GALLLALAIL GGLSKLHCTR  173

GLP1R  177  NYIHLNLFAS FILRALSVFI KDAALKWMYS -TAAQQHQWD GLLSYQDSLS CRLVFLFLMQY  235
GCGR   174  NAIHANLFAS FVLKASSVLV IDGLLRTRYS QKIGDDLSVS TWLSDGAVAG CRVAAVFMQY  233

GLP1R  236  CVAANYYWLL VEGVYLYTLL AFSVFSEQWI FRLYVSIGWG VPLLFVVPWG IVKYLYEDEG  295
GCGR   234  GIVANYCWLL VEGLYLHNLL GLATLPERSF FSLYLGIGWG APMLFVVPWA VVKCLFENVQ  293

GLP1R  296  CWTRNSNMNY WLIIRLPILF GIGVNFLIFV RVICIVVSKL KANLMCKTDI KCRLAKSTLT  355
GCGR   294  CWTSNDNMGF WWILRFPVFL AILINFFIFV RIVQLLVAKL RARQMHHTDY KFRLAKSTLT  353

GLP1R  356  LIPLLGTHEV IFAFVMDEHA RGTLRFIKLF TELSFTSFQG LMVAILYCFV NNEVQLEFRK  415
GCGR   354  LIPLLGVHEV VFAFVTDEHA QGTLRSAKLF FDLFLSSFQG LLVAVLYCFL NKEVQSELRR  413

GLP1R  416  SWERWRLEHL HIQ------- RDSSMKPLKC PTSSLSSGAT AGSSMYTATC QASCS-----  463
GCGR   414  RWHRWRLGKV LWEERNTSNH RASSSPGHGP PSKELQFGRG GGSQDSSAET PLAGGLPRLA  473

GLP1R  463  ----  463
GCGR   474  ESPF  477
```

B.
Clustal W alignment of GCGR peptide sequence with GLP2R peptide sequence.

```
GLP2R  1    MKLGSSRAGP GRGSAGLLPG VHELPMGIPA PWGTSPLSFH RKCSLWAEGR PPLTLVLLVS  60
GCGR   1    ---------- ---------- -------MPP ---------- -----CQPQR PLLLLLLLLA  18

GLP2R  61   IK-QVT-GSL LEETTRKWAQ YKQACLR--D LLKEPSGIFC NGTFDQYVCW PHSSPGNVS-  115
GCGR   19   CQPQVPSAQV MDFLFEKWKL YGDQCHHNLS LLPPPTELVC NRTFDKYSCW PDTPANTTAN  78

GLP2R  116  VPCPSYLPWW SEESSGRAYR HCLAQGTWQT IENATDIWQD DSECSEN-HS FKQNVDRYAL  174
GCGR   79   ISCPWYLPWH HKVQHRFVFK RCGPDGQWVR GPRG-QPWRD ASQCQMDGEE IEVQKEVAKM  137

GLP2R  175  ISTLQLMYTV GYSFSLISLF LALTLLLFLR KLHCTRNYIH MNLFASFMLR TLAVLVKDVV  234
GCGR   138  YSSFQVMYTV GYSLSLGALL LALAILGGLS KLHCTRNAIH ANLFASFVLK ASSVLVIDGL  197

GLP2R  235  FYNSYSKRPD NENGWMSYLS EMSTS-CRSV QVLLHYFVGA NYLWLLVEGL YLHTLLEPTV  293
GCGR   198  LRTRYSQKIG DDLSVSTWLS DGAVAGCRVA AVFMQYGIVA NYCWLLVEGL YLHNLLGLAT  257

GLP2R  294  LPERRLWPRY LLLGWGAPVL FVVPWGFARA HLENTGCWTT NGNKKIWWIL RGPMMLCVTV  353
GCGR   258  LPERSFFSLY LGIGWGAPML FVVPWAVVKC LFENVQCWTS NDNMGFWWIL RFPVFLAILI  317

GLP2R  354  NFFIFLKILK LLISKLRAHQ MCFRDYKYRL AKSTLVLIPL LGVHEILFSF ITDDQVFGFA  413
GCGR   318  NFFIFVRIVQ LLVAKLRARQ MHHTDYKFRL AKSTLTLIPL LGVHEVVFAF VTDEHAQGTL  377

GLP2R  414  KLIRLFIQLT LSSFHGDLVA LQYGFANGEV KAELRKYWVR FLLARHSGCR ACVLGKDFRF  473
GCGR   378  RSAKLFFDLF LSSFQGLLVA VLYCFLNKEV QSELRRWHR WRLG------ ----------  421

GLP2R  474  LGKCPKKLSE GDGAEKLRKL QPSLNSGRLL HLAMPGLGEL GAQPQQDHAR WPRGSSLSEC  533
GCGR   421  ---------- ----KVLWEE RNTSN----- ---HRASSSP GHGPPSKELQ FGRGGGSQDS  459

GLP2R  534  SEGDVTMANT MEEILPESEI  553
GCGR   460  S-AETPLAGG LPRLAESPF-  477
```

Figure 7 (continued)

C.

Alignment of Glucagon, Glucagon related peptide 1 (GLP1) and Glucagon related peptide (GLP2).

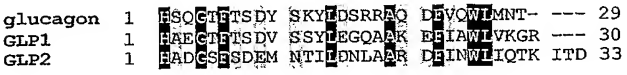


Figure 8

A.
Clustal W alignment of FPRL1 peptide sequence with FPR1 peptide sequence.

```
FPRL1   1   METNFSTPLN EYEEVSYESA GYTVIRILPL VVLGVTFVLG VLGNGLVIWV AGFRMTRTVT  60
FPR1    1   METNSSLPTN ISGGTPAVSA GYLFLDIITY LVFAVTFVLG VLGNGLVIWV AGFRMTHTVT  60

FPRL1  61   TICYLNLALA DFSFTATLPF LIVSMAMGEK WPFGWFLCKL IHIVVDINLF GSVFLIGFIA  120
FPR1   61   TISYLNLAVA DFCFTSTLPF FMVRKAMGGH WPFGWFLCKF VFTIVDINLF GSVFLIALIA  120

FPRL1 121   LDRCICVLHP VWAQNHRTVS LAMKVIVGPW ILALVLTLPV FLFLTTVTIP NGDTYCTFNF  180
FPR1  121   LDRGVCVLHP VWTQNHRTVS LAKKVIIGPW VMALLLTLPV IIRVTTVPGK TGTVACTFNF  180

FPRL1 181   ASWGGTPEER LKVAITMLTA RGIIRFVIGF SLPMSIVAIC YGLIAAKIHK KGMIKSSRPL  240
FPR1  181   SPWTNDPKER INVAVAMLTV RGIIRFIIGF SAPMSIVAVS YGLIATKIHK QGLIKSSRPL  240

FPRL1 241   RVLTAVVASF FICWFPFQLV ALLGTVWLKE MLFYGKYKII DILVNPTSSL AFFNSCLNPM  300
FPR1  241   RVLSFVAFAF FLCWSPYQVV ALIATVRIRE LLQGMYKEI GIAVDVTSAL AFFNSCLNPM  299

FPRL1 301   LYVFVGQDFR ERLIHSLPTS LERALSEDSA PTNDTAANSA SPPAETELQA M 351
FPR1  300   LYVFMGQDFR ERLIHALPAS LERALTEDST QTSDTATNST LPSAEVELQA K 350
```

Identical residues 71% (background black)
Similar residues 82% (background gray)

B. Clustal W alignment of FPRL1 peptide sequence with FPRL2 peptide sequence.

```
FPRL1   1   METNFSTPLN EYEEVSYESA GYTVIRILPL VVLGVTFVLG VLGNGLVIWV AGFRMTRTVT  60
FPRL2   1   METNFSIPLN ETEEVLPEPA GHTVIWIFSL LVHGVTFVFG VLGNGLVIWV AGFRMTRTVN  60

FPRL1  61   TICYLNLALA DFSFTATLPF LIVSMAMGEK WPFGWFLCKL IHIVVDINLF GSVFLIGFIA  120
FPRL2  61   TICYLNLALA DFSFSAILPF RMVSVAMRER WPFASFLCKL VHVMIDINLF VSVYLITLIA  120

FPRL1 121   LDRCICVLHP VWAQNHRTVS LAMKVIVGPW ILAIVLTLPV FLFLTTVTIP NGDTYCTFNF  180
FPRL2 121   LDRCICVLHP AWAQNHRTMS LAKRVMTGLW IFTIVLTLPN FIFWTTISTT NGDTYCIFNF  180

FPRL1 181   ASWGGTPEER LKVAITMLTA RGIIRFVIGF SIPMSIVAIC YGLIAAKIHK KGMIKSSRPL  240
FPRL2 181   PFWGDTAVER LNVFITMAKV FLILHFIIGF TVPMSIITVC YGIIAAKIHR NHMIKSSRPL  240

FPRL1 241   RVLTAVVASF FICWFPYQLV ALLGTVWLKE MLFYGKYKII DILVNPTSSL AFFNSCLNPM  300
FPRL2 241   RVFAAVVASF FICWFPYELI GILMAVWLKE MLLNGKYKII LVLINPTSSL AFFNSCLNPI  300

FPRL1 301   LYVFVGQDFR ERLIHSLPTS LERALSE--D SAPTNDTAAN SASPPAETEL QAM 351
FPRL2 301   LYVFMGRNFQ ERLIRSLPTS LERALTEVPD SAQTSNTHTT SASPPETETL QAM 353
```

Identical residues 68% (background black)
Similar residues 78% (background gray)

Figure 9

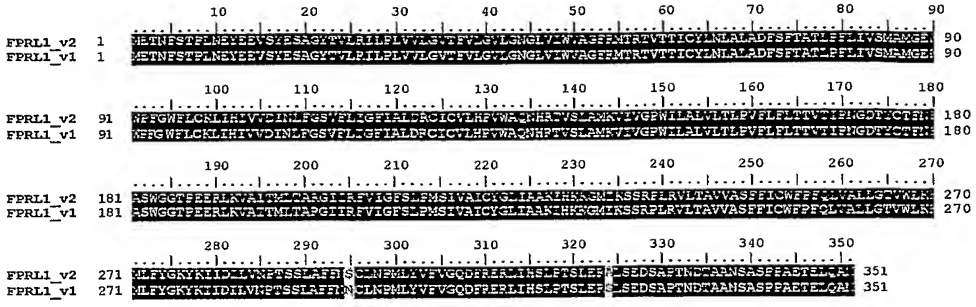


Figure 10

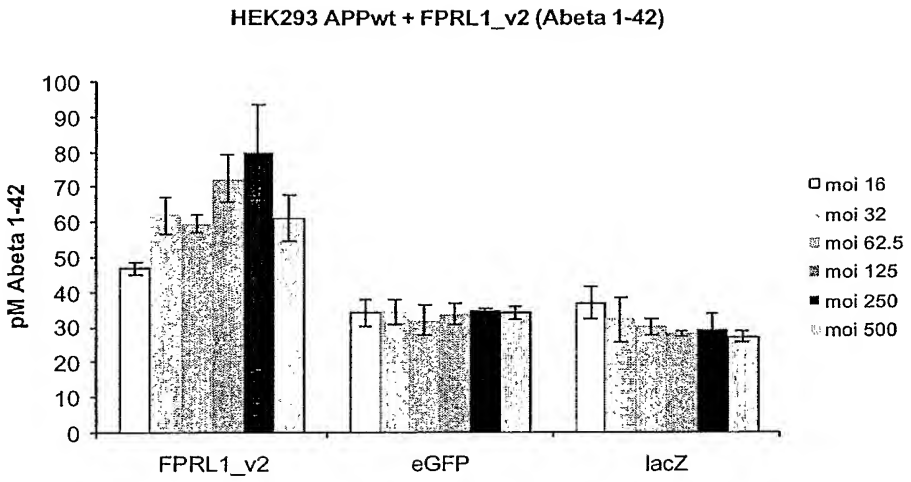

HEK293 APPwt + FPRL1_v2 (Abeta 1-42)
pM Abeta 1-42
100
90
80
70
60
50
40
30
20
10
0
FPRL1_v2
eGFP
lacZ
moi 16
moi 32
moi 62.5
moi 125
moi 250
moi 500

Figure 11

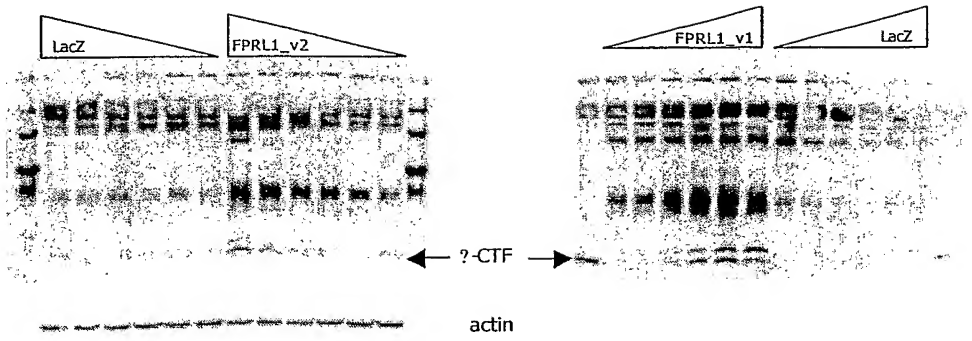


Figure 12

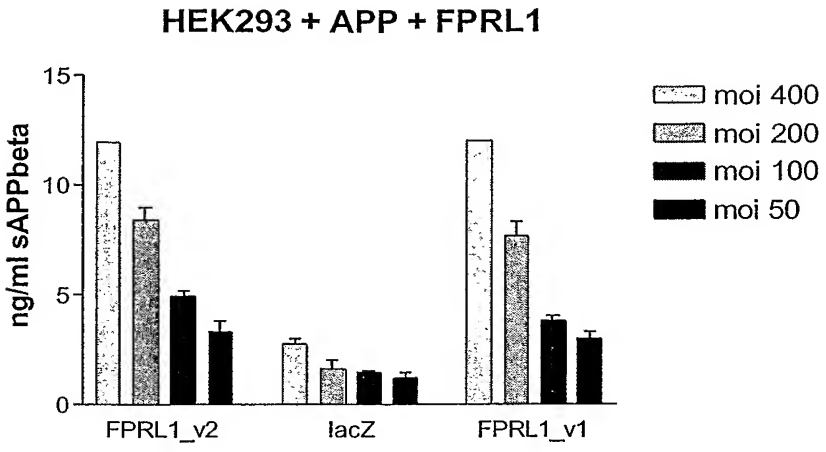
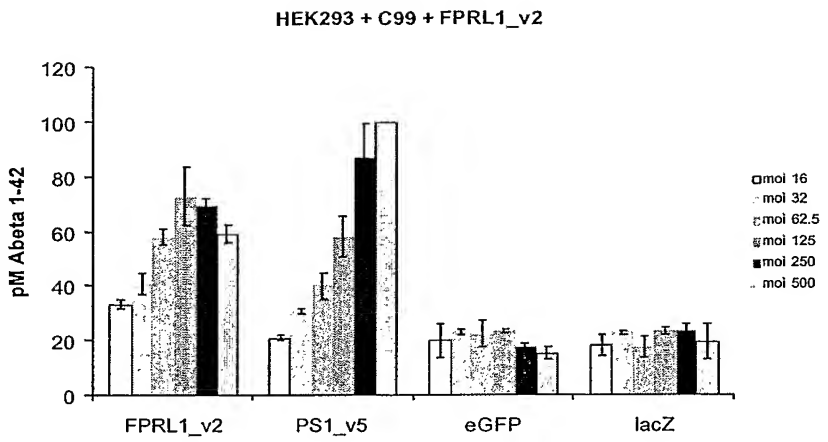

HEK293 + APP + FPRL1
ng/ml sAPPbeta
15
10
5
0
FPRL1_v2
lacZ
FPRL1_v1
moi 400
moi 200
moi 100
moi 50

Figure 13

A.


HEK293 + C99 + FPRL1_v2
pM Abeta 1-42
120
100
80
60
40
20
0
FPRL1_v2
PS1_v5
eGFP
lacZ
moi 16
moi 32
moi 62.5
moi 125
moi 250
moi 500

B.

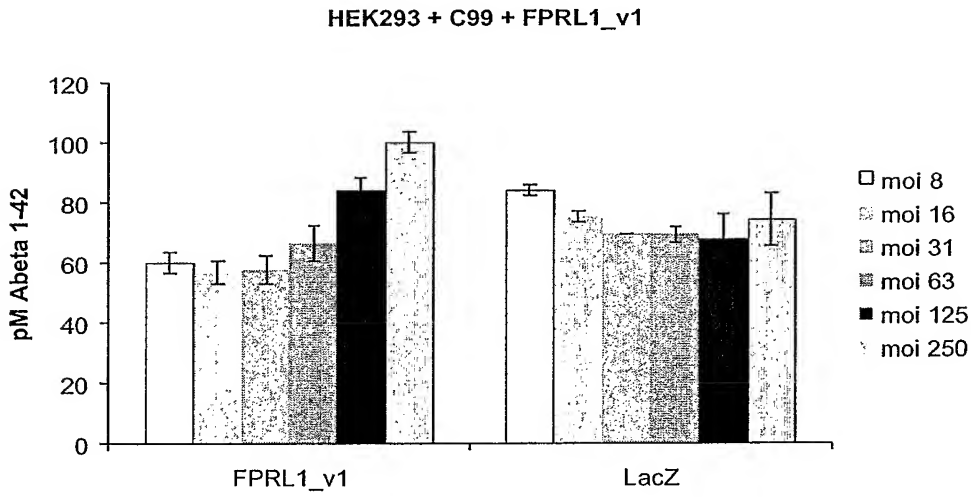

HEK293 + C99 + FPRL1_v1
pM Abeta 1-42
120
100
80
60
40
20
0
FPRL1_v1
LacZ
moi 8
moi 16
moi 31
moi 63
moi 125
moi 250

Figure 14

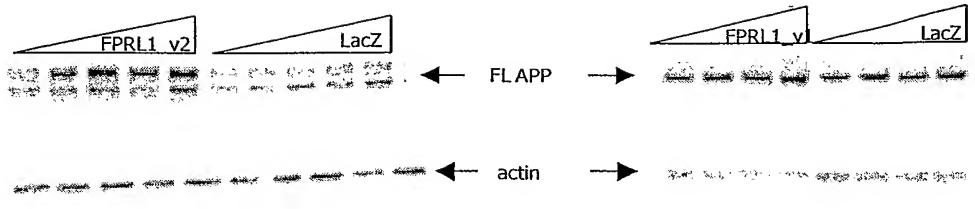


Figure 15

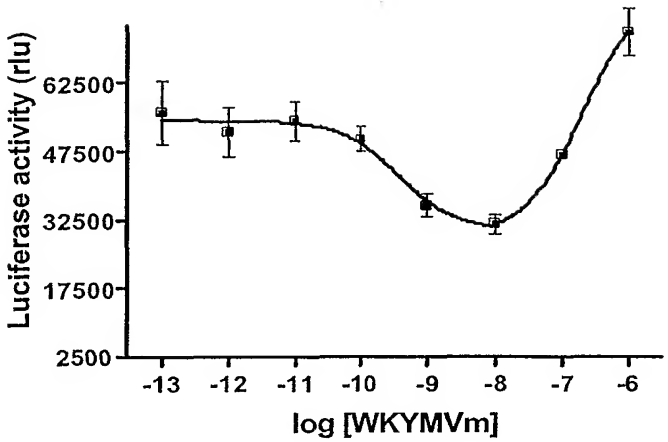

Luciferase activity (rlu)
62500
47500
32500
17500
2500
-13
-12
-11
-10
-9
-8
-7
-6
log [WKYMVm]

Figure 16

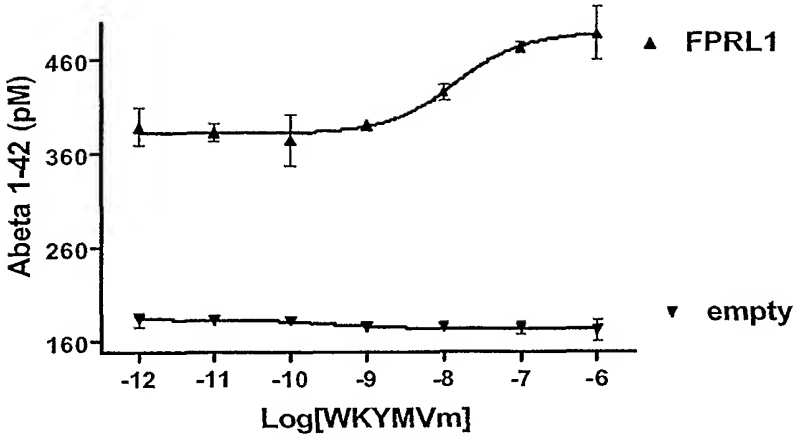

Abeta 1-42 (pM)
460
360
260
160
-12
-11
-10
-9
-8
-7
-6
Log[WKYMVm]
FPRL1
empty

Figure 17

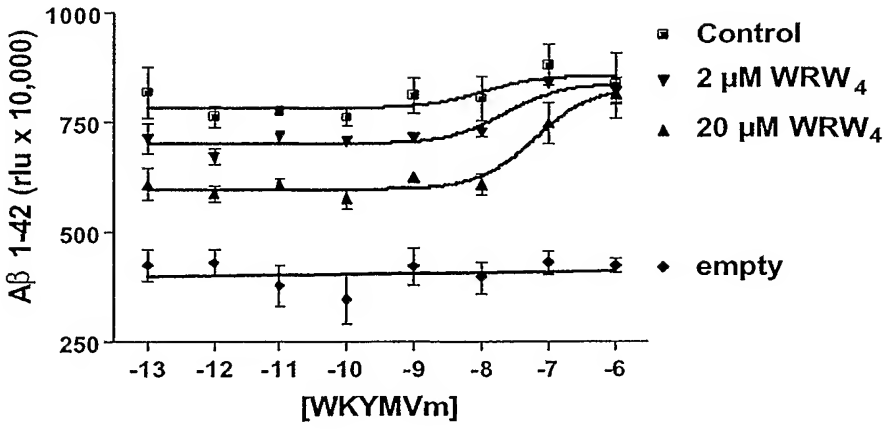
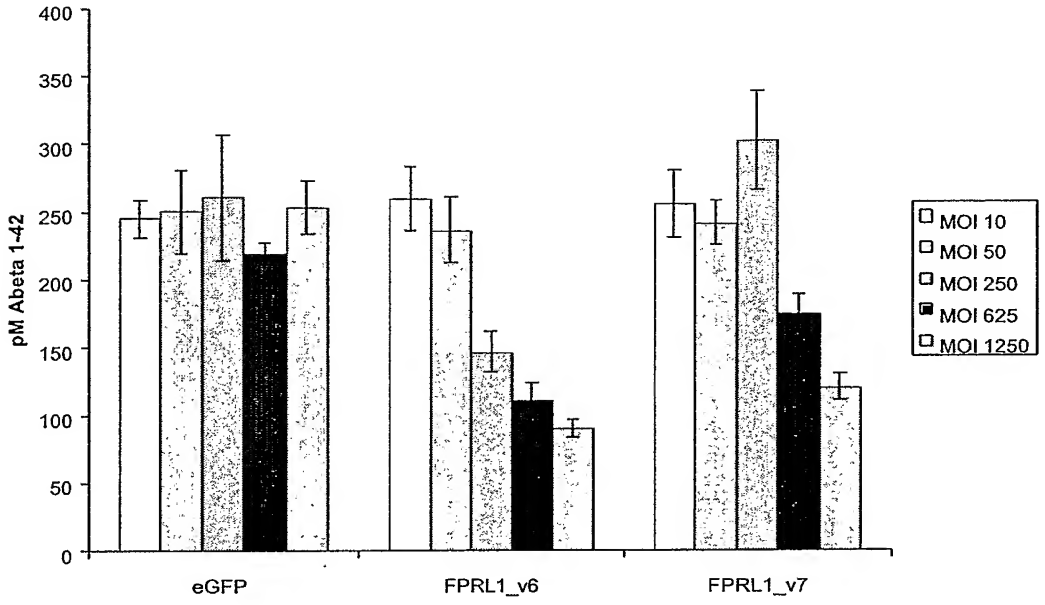

1000
750
500
250
Aβ 1-42 (rlu x 10,000)
-13
-12
-11
-10
-9
-8
-7
-6
[WKYMVm]
Control
2 µM WRW4
20 µM WRW4
empty

Figure 18


400
350
300
250
200
150
100
50
0
pM Abeta 1-42
eGFP
FPRL1_v6
FPRL1_v7
MOI 10
MOI 50
MOI 250
MOI 625
MOI 1250